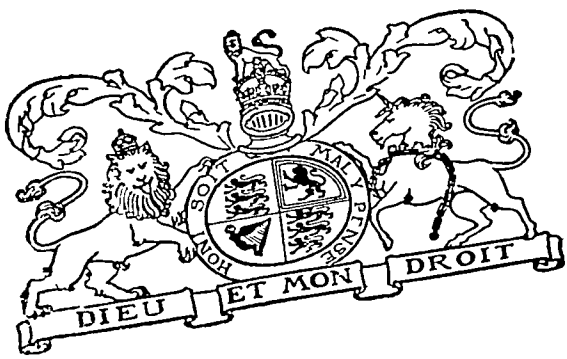

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Editor —Lieut Colonel J TAYLOR, D S O, M D., D P H., I M S.,
Director, Central Research Institute, Kasauli

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ON THE STANDARDIZATION OF RUSSELL'S VIPER ANTIVENIN

BY

LIEUT-COLONEL L A P ANDERSON, M A, M D, D P H, D T M & H, F Z S I M S.

CORRIGENDA

In the *Indian Medical Record* No 21 The Life Line of the Thyroid Gland' by Colonel P McCarrison C I E I M S and Mr K B Madhava M A A M S I add the following corrections —

On pages 7 and 119 for noxia *read* nova

On page 101 1st column of the Table for '0 01 : 0 11' *read* 10 01 : 0 11

On page 124, 4th column of the Table for '6 8 : 1 16 *read* '16 8 : 1 16'

On page 111 penultimate line for '8 out of 100 cases' *read* '8 out of 10 cases'

On page 17 Table at the bottom, 'Number of Goitres' in the 4th column refers to Summer and not to Winter' The dividing line is in the wrong place

On page 223, 4th line for 'Fig 20(a) and' *read* 'Figs 20(a) and 20(b)'

because the Kasauli serum is [illegible] partly because these snakes are two of the more common poisonous snakes of India and consequently their venoms would reasonably be expected to be neutralized by 'Serum A V'

In the routine standardization test carried out on all batches of Kasauli antivenin before issue, the neutralizing value of the serum against cobra venom only is estimated This is done by a biological test which offers no special difficulties and the average neutralizing value of the standard serum as regards cobra venom, both *in vitro* and *in vivo*, is well known to any worker in the Serum Department of the Central Research Institute This is not the case, however, as regards its value against daboia venom Since the early work of Lamb and his co-workers, carried out between the years 1899 and 1905, no serious attempt had been made to

determine a reliable method of standardizing a serum specifically active against daboia venom until a series of papers on snake venoms and the treatment of snake bite were published by Acton and Knowles between the years 1913 and 1915 It may safely be said that the anti-daboia value of the Kasauli serum was quite unknown

Previous work

The pioneer work on the venoms and antivenins of the common Indian snakes is undoubtedly that of Lamb and his associates, who published their results from time to time in the Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India Much of this valuable work is in danger of being forgotten and little reference to it can be found in the writings of recent workers on this subject, largely, no doubt, owing to the inaccessibility of the papers

Although Lamb and his co-workers carried out a very extensive investigation into the properties of venoms and antivenins, there are, however, comparatively few references to actual tests to determine the neutralizing power of specific daboia antivenins and much of their work dealing with the neutralization of daboia venom was carried out with sera prepared from the venoms of other varieties of snakes, in the course of their studies of the specificity of venoms and antivenins generally This work, however, brought to light many facts as regards the constitution and action of venoms and their interaction with specific sera, which were hitherto insufficiently understood and which are really the basis of all subsequent work on the standardization of antivenomous sera

Acton and Knowles (1913, 1914 and 1915) carried out an investigation on the treatment of snake-bite and allied problems, including a study of Indian snake venoms and the action of various antivenomous sera, and in the course of this they considered the question of standardization of antivenin The method they employed, so far as the neutralizing power of antivenins *in vitro* is concerned, gave consistent results probably closely approximating to the actual potency of the sera They called attention to the fallacy of ignoring the minimum lethal dose of the venom for the particular animal used, in estimating the amount of venom neutralized by the serum, a point on which Semple and Lamb (1899) had originally laid stress

As regards work with daboia venom and antivenin, which is the particular subject we are concerned with here, Lamb and Hanna (1903) noted that this venom, whose toxic effect is principally on the vascular system, has a double action, causing on the one hand thrombosis and on the other hæmorrhages, according to the dose and the route by which it is administered Thus, given direct into the blood stream, or, if in sufficiently large doses subcutaneously, rapid death due to intravascular thrombosis occurred A dose given subcutaneously, though insufficient to cause death by thrombosis, might still, however, produce chronic

intoxication and slower death with prominent signs of hæmorrhage and œdema Further they observed that an intravascular dose insufficient to cause immediate death by thrombosis produced no, or very slight, symptoms

Putting these observations into practice, Lamb and Hanna (1903) and subsequently Lamb (1903 and 1905) carried out several small scale experiments to determine the neutralizing effect of various sera on daboia venom

As regards the neutralizing power *in vitro*, having ascertained the intravenous M L D of the venom for the rabbit, that is, the amount just sufficient to cause death by intravascular clotting a multiple of the M L D was mixed with varying quantities of serum and the mixture after being allowed to stand for some time was injected into the marginal ear vein Unfortunately only one experiment is recorded (Lamb, 1905) in which a specific anti-daboia serum was tested against daboia venom All the other experiments dealt with heterologous sera and were negative as regards any neutralizing effect The one positive experiment, however, showed the possibilities of the method

Only one experiment is recorded in which the neutralizing effect *in vivo* was investigated This too concerned a heterologous serum and was completely negative (Lamb, 1905) In this experiment, rabbits again being used, the serum was injected into one ear vein five minutes before the test dose of venom was given into the other ear vein The dose of venom was, as before, a multiple of the M L D The reason for injecting the serum and venom in this order is, of course, obvious

Acton and Knowles (1914) attempted to determine the minimum lethal dose of daboia venom by subcutaneous injection in rats and monkeys and they remark on the difficulty of estimating this with certainty using this route, probably, as they say, owing to local thrombosis

Previously these same workers (Acton and Knowles, 1913) had determined the neutralizing value against daboia venom of certain anti-daboia sera *in vitro*, using rabbits In this work they employed essentially the same method as that used before by Lamb and Lamb and Hanna, with the difference that a fixed quantity of serum was mixed with varying amounts of venom Though they do not specifically mention this fact, they estimated the M L D of the venom as that quantity which was just sufficient to kill by intravascular thrombosis They remark that the results of such experiments *in vitro* are unlikely to indicate the neutralizing value of the serum under conditions such as are met with in actual snake-bite and suggested that experiments *in vivo* using animals more closely allied to man would be of greater value

Accordingly, later on (Acton and Knowles, 1915), they carried out a short series of experiments on monkeys in which the venom and serum were administered separately, the latter by different routes, so that the toxin and anti-toxin came in contact for the first time in the animal body, as would be the case in actual

snake-bite In this work a fixed dose of venom was given subcutaneously and followed immediately by a dose of serum, varying with each experiment, given subcutaneously, intraperitoneally or intravenously

Although these experiments are of considerable value as indicating what may be expected to be the result of serum therapy in cases of Russell's viper bite, they do not of course give the true neutralizing value of the serum *in vivo* and from this aspect cannot be compared with the previous *in vitro* tests as a means of standardizing the serum Owing to the irregularity with which daboia venom is absorbed after subcutaneous injection, it is quite impossible to say how much of the test dose was absorbed in any one experiment, while for the same reason it is not admissible to estimate the potency of the serum in terms of the subcutaneous M L D , as they necessarily had to do in this case Indeed Acton and Knowles themselves here again call attention to this fallacy This work, however, was primarily carried out to compare the value of the various methods of administering the serum and not directly as a method of standardization

In all the above work, both of Acton and Knowles as also of Lamb and his associates, the M L D or the estimated sub-lethal dose of the venom was deducted in calculating the amount of venom neutralized by the serum

Taking all the above facts into consideration it is apparent that in order to determine the actual neutralizing power of a serum against daboia venom by any biological method, whether *in vitro* or *in vivo*, for standardization purposes only and leaving aside the question of estimating its probable therapeutic value in snake-bite, it is desirable to give all injections intravascularly As a natural corollary to this the M L D of the venom on which the standardization is based, would be estimated as the smallest dose just capable of causing rapid death by intravascular clotting , repeated experiments, amply confirmed by the present writer, have shown that a dose even fractionally smaller than this causes no symptoms

Experimental work

The whole investigation carried out by the present writer involved a very large series of experiments in which the neutralizing value of 23 samples of antivenomous serum was determined, both *in vitro* and *in vivo*, against both cobra and Russell's viper venoms Over 50 separate experiments, requiring the use of nearly 400 animals, were conducted, comprising the actual neutralizing tests together with tests to determine the minimum lethal doses of the venoms used and the necessary venom and serum controls We are at present concerned, however, particularly with the standardization of sera against daboia venom so that only those experiments which relate to this part of the work will be considered here

Throughout this work pigeons were the animals used For reasons which will be referred to later these animals seem particularly suitable for this work, and apart from this they are easily obtainable in large numbers, cheap initially and to

feed, reasonably constant in weight and easy to inoculate intravenously So far as possible birds of about 300 grm weight were used and only those in good condition were taken Every pigeon was weighed before each experiment

The sera tested were (1) nine samples of standard Kasauli antivenin varying in age from a few months to 3 years and stored in different places in India under varying conditions The standard Kasauli serum is prepared from horses immunized with the mixed venoms of cobra and Russell's viper (*Daboia Russellii*) and contains no preservative, being drawn and bottled with strict aseptic precautions It is put up in bottles of 10 c c The serum is standardized, 1 c c neutralizing at least 0 5 mg of cobra venom The neutralizing power as regards daboia venom was unknown, but the average value as regards cobra venom of the fresh serum is such that 1 c c neutralizes from 0 8 to 1 0 mg The fresh serum is quite clear but the older samples contain a heavy deposit increasing with age The serum is regarded as time expired after two years (2) Fourteen samples of 'Serum A V' This was purchased from a commercial firm It was received in sealed capsules of 10 c c , each bearing a control number The serum was a thin, slightly yellowish, faintly opalescent fluid It was said to be effective against the bites of Indian snakes (one capsule bearing the same control number was contained in a box whose label stated that the serum was suitable for the treatment of bites by African snakes) Nothing was known definitely about the method of preparation of this serum nor about the venoms from which it had been prepared Its age was also unknown, but as it was being sold in the open market it was presumably not regarded as time expired

The venom used was the dried venom of the Russell's viper It was the pooled samples from several snakes of this species which are 'milked' at the Haffkine Institute, Bombay, where the serum is dried, put up in sealed tubes and sent to Kasauli for the preparation of antivenin In appearance the dried venom is a light yellow (usually) to darkish green (rarely) crystalloid substance It is readily soluble in the cold in distilled water or in physiological salt solution, forming an opalescent solution

Preliminary tests

Every bottle of serum before being used for the neutralization experiments was tested in a vacuum chamber for the sealing, and by aerobic and anaerobic culture for sterility Every bottle passed these tests satisfactorily

Preparation of the venom solution

In all experiments to be detailed below, the venom solutions were prepared as follows The dried venom was accurately weighed on a chemical balance (Sartorius) to the nearest $\frac{1}{10}$th mg and dissolved in sterile physiological salt solution to make a solution containing 10 (sometimes 5) mg per 1 c c From this, further serial

dilutions in physiological salt solution were prepared containing in 1 c c the various doses of venom required for the day's tests All the solutions were freshly prepared immediately before each day's tests and any material left over was discarded

'Record' syringes were used for all the injections

Determination of the minimum lethal dose of daboia venom for pigeons

(1) *By intramuscular injection* —In the hope that neutralization tests on the lines followed in those with cobra venom might be possible in the case of daboia venom also, and in ignorance of the results of previous workers, some time was wasted in attempting to determine the M L D by the intramuscular route The venom was injected into the breast muscles Table I shows the result of one such experiment and confirms the findings of the earlier workers referred to above

Table I

Daboia venom Determination of the minimum lethal dose by intramuscular injection Pigeons

One c c of venom solution containing various doses, injected into the breast muscles Results read after 24 hours

Date of test	Pigeon number	Weight in grammes	Test dose	Results
6-9-1923	1	320	1 2 mg daboia venom	Died within 24 hours
	2	About 300 each	1 4 " " "	Alive and well
	3		1 6 " " "	" " "
	4	300	1 8 " " "	Died in 45 minutes
	5	310	2 0 " " "	" " 2 hours

(2) *By intravenous injection* —After this preliminary failure the determination of the M L D intravenously was tried, the dose of venom being injected directly into the large wing vein The results of three of these experiments are given in Table II This table shows how easily the M L D of this particular sample of venom was determined and the degree of regularity obtained Throughout the whole of this series of experiments, in each of which a 'Venom Control' was included, the M L D of this sample of venom never varied more than ± 0 01 mg and that but rarely This experiment also confirmed the observation of Lamb and Hanna (1903), namely, that death when it occurred was almost immediate and that a dose just below the M L D produced no symptoms

TABLE II

Daboia venom Determination of the minimum lethal dose by intravenous injection Pigeons

One c c of venom solution containing various doses, injected into the wing vein
Results read after 24 hours

Date of test	Pigeon number	Weight in grammes	Test dose	Results	REMARKS
6-9-1923	6	340	0 4 mg daboia venom	All died	Death occurred in convulsions almost immediately after the injection
	7	260	0 6 „ „ „		
	8	270	0 8 „ „ „		
	9	270	0 6 „ „ „		
10-9-1923	10	All between 250 and 320	0 005 mg daboia venom	Alive and well	Where death occurred, it was almost immediate
	11		0 01 „ „ „	„ „ „	Otherwise there were no symptoms
	12		0 02 „ „ „	„ „ „	The M L D is 0 03 mg
	13		0 03 „ „ „	Died	
13-9-1923	14	All between 250 and 320	0 005 mg daboia venom	Alive and well	As before, death from a lethal dose was almost immediate
	15		0 01 „ „ „	„ „ „	
	16		0 02 „ „ „	„ „ „	The M L D is 0 03 mg
	17		0 03 „ „ „	Died	

L. A. P. Anderson

THE NEUTRALIZATION OF DABOIA VENOM *in vitro*

For these tests the technique was essentially the same as that employed by Acton and Knowles One c c of the venom solution (containing the various test doses) was mixed with 1 c c of the serum, kept at 37°C for half an hour and the mixture (2 c c) then injected into the wing vein With each series of tests an M L D control for the venom was put up, the dose of venom in 1 c c solution being injected into the wing vein In estimating the amount of venom neutralized by the serum the M L D of the venom, as shown by that day's venom control, was deducted The results could be read immediately, since the birds either died almost at once or showed no obvious symptoms, in practice, however, the final result was not recorded until after 24 hours in case death should be delayed In actual fact, on no occasion did any of the birds injected and which survived the immediate effect of the venom exhibit any signs of poisoning and no delayed deaths occurred

Table III gives the results obtained with a number of different sera

It will be seen from this table that this method gives remarkably regular results and that the neutralizing point is usually quite clear cut The table also shows that a relatively fresh serum, stored in good conditions, has a considerable potency against daboia venom, considerably greater, in fact, than that obtainable against cobra venom Thus, sera Nos C R I 427, 462, 481 and 421, none of which was more than 1 year old and all stored in the relatively cool climate of Kasauli or in an ice box in the plains, neutralized, in 1 c c, at least 2 47, 2 47, 1 97 and 1 47 mg of daboia venom respectively The amount of cobra venom neutralized by these same sera, estimated during the course of this investigation, was respectively 0 3, 0 5, 0 5 and 0 2 mg

The potency of these sera agrees very fairly with that found independently by other workers, using different samples of anti-daboia serum For example Acton and Knowles (1913) estimated the neutralizing value, *in vitro*, using rabbits, of certain anti-daboia sera and found values for 1 c c equal to 1 98 and 1 8 mg Lamb (1905) standardized by the *in vitro* test an antivenin prepared from pure daboia venom, using a slightly different method, and found that 1 c c neutralized 2 8 mg of daboia venom He also observed that the potency of cobra antivenins is usually much lower, the strongest he had been able to prepare had a value of 1 c c equal to 1 5 mg, but they were usually much less potent

One rather striking difference between the present experiments and those of previous workers must be noted In carrying out their neutralizing tests *in vitro* both Lamb (1905) and Acton and Knowles (1913) observed cases in which the animals (rabbits) given intravenously mixtures of serum and venom either showed marked symptoms and survived or died after a more or less prolonged period, although the control animals given venom alone, or venom plus saline, as a rule died almost at once or showed no symptoms This effect appears to have been

TABLE III

The neutralizing power of various antivenomous sera against daboia venom in vitro *Pigeons*

One c c of the serum mixed with one c c of daboia venom solution (containing various concentrations of venom) Mixture kept in the incubator at 37°C for half an hour and then injected into the wing vein Results read after 24 hours

Venom control doses injected intravenously

Date	Serum and number	Pigeon number	Weight in grammes	Test dose	Results	Remarks
24-9-1923	C R I 462	33	280	1 c c serum plus 0 5 mg venom	All alive and well	1 c c serum neutralizes 0 96 mg daboia venom or more (upper limit not reached by the experiment)
		34	280	1 „ „ „ 0 6 „ „		
		35	280	1 „ „ „ 0 7 „ „		
		36	300	1 „ „ „ 0 8 „ „		
		37	320	1 „ „ „ 0 9 „ „		
		38	280	1 „ „ „ 1 0 „ „		
24-9-1923	'Serum A V' No 5/5	39	300	1 c c serum plus 0 5 mg venom	All died	1 c c serum neutralizes less than 0 46 mg daboia venom (lower limit not obtained by the experiment)
—		40	280	1 „ „ „ 0 6 „ „		
		41	290	1 „ „ „ 0 7 „ „		
		42	280	1 „ „ „ 0 8 „ „		

TABLE III—*contd*

Date	Serum and number	Pigeon number	Weight in grammes	Test dose	Results	REMARKS
24-9-1923	Daboia venom control	43	300	0 01 mg venom	Alive and well	The M L D is 0 04 mg
		44	320	0 02 „ „	„ „ „	
		45	300	0 03 „ „	„ „ „	
		46	300	0 04 „ „	Died	
11-10-1923	'Serum A V' No 9/1	119	270	1 c c serum plus 0 04 mg venom	Alive and well	1 c c serum neutralizes 0 03 mg daboia venom and may neutralize 0 04 mg
		120	270	1 „ „ „ 0 06 „ „	„ „ „	
		121	270	1 „ „ „ 0 08 „ „	Died	
		122	270	1 „ „ „ 0 10 „ „	„	
		123	270	1 „ „ „ 0 20 „ „	„	
11-10-1923	'Serum A V' No 5/2	124	280	1 c c serum plus 0 04 mg venom	Alive and well	1 c c serum neutralizes 0 01 mg daboia venom and may neutralize 0 02 mg
		125	280	1 „ „ „ 0 06 „ „	Died	
		126	280	1 „ „ „ 0 08 „ „	„	
		127	280	1 „ „ „ 0 10 „ „	„	
		128	300	1 „ „ „ 0 20 „ „	„	

11-10-1923	C R I 462	129	300	1 c c serum plus 0 9 mg venom	All alive and well	1 c c serum neutralizes 1 97 mg daboia venom or more (upper limit not reached by the experiment)
		130	300	1 „ „ „ 1 0 „ „		
		131	290	1 „ „ „ 1 5 „ „		
		132	290	1 „ „ „ 2 0 „ „		
11-10-1923	C R I 427	133	300	1 c c serum plus 0 5 mg venom	All alive and well	1 c c serum neutralizes 1 97 mg daboia venom or more (upper limit not reached by the experiment)
		134	300	1 „ „ „ 0 6 „ „		
		135	300	1 „ „ „ 0 7 „ „		
		136	300	1 „ „ „ 0 8 „ „		
		137	300	1 „ „ „ 0 9 „ „		
		138	280	1 „ „ „ 1 0 „ „		
		139	300	1 „ „ „ 1 5 „ „		
		140	280	1 „ „ „ 2 0 „ „		
11-10-1923	C R I 252	141	310	1 c c serum plus 0 3 mg venom	Alive and well	No 147 pigeon apparently survived by accident 1 c c serum neutralizes between 0 57 and 0 66 mg daboia venom This serum was time expired
		142	300	1 „ „ „ 0 4 „ „	„ „	
		143	290	1 „ „ „ 0 5 „ „	„ „ „	
		144	250	1 „ „ „ 0 6 „ „	„ „ „	
		145	280	1 „ „ „ 0 7 „ „	Died	
		146	260	1 „ „ „ 0 8 „ „	„	
		147	310	1 „ „ „ 0 9 „ „	Alive and well	
		148	300	1 „ „ „ 1 0 „ „	Died	

TABLE III—*concld*

Date.	Serum and number	Pigeon number	Weight in grammes	Test dose	Results	REMARKS
11-10-1923	Daboia venom control	149	310	0 01 mg venom	Alive and well	The M L D is 0 03 mg
		150	310	0 02 „ „	„ „ „	
		151	300	0 03 „ „	Died	
		152	300	0 04 „ „	„	
15-11-1923	C R I 427	153	320	1 c c serum plus 2 0 mg venom	Alive and well	1 c c serum neutralizes between 2 47 and and 2 96 mg daboia venom
		154	320	1 „ „ „ 2 5 „ „	„ „ ,	
		155	280	1 „ „ „ 3 0 „ „	Died	
		156	280	1 „ „ 3 5 ,	„	
15-10-1923	C R I 462	157	290	1 c c serum plus 2 0 mg venom	Alive and well	1 c c serum neutralizes between 2 47 and 2 96 mg daboia venom
		158	290	1 „ „ „ 2 5 „ „	, „ „	
		159	270	1 „ „ „ 3 0 „ „	Died	
		160	310	1 „ „ „ 3 5 „ „	„	

15–10–1923	Daboia venom control	161	300	0 02 mg venom	Alive and well	The M L D is 0 03 mg
		162	310	0 03 „ „	Died	
		163	310	0 04 „ „	„	
25–10–1923	C R I 481	212	340	1 c c serum plus 2 0 mg venom	Alive and well	1 c c serum neutralizes between 1 97 and 2 16 mg daboia venom
		213	290	1 „ „ „ 2 5 „ „	Died	
		214	300	1 „ „ „ 3 0 „ „	„	
		215	280	1 „ „ „ 3 5 „ „	„	
25–10–1923	C R I 421	216	280	1 c c serum plus 1 0 mg venom	Alive and well	1 c c serum neutralizes between 1 47 and 1 96 mg daboia venom
		217	310	1 „ „ 1 5 „ „	„ „ „	
		218	280	1 „ „ „ 2 0 „ „	Died	
		219	300	1 „ „ „ 2 5 „ „	„	
		220	280	1 „ „ „ 3 0 „ „	„	
25–10–1923	Daboia venom control	236	300	0 02 mg venom	Alive and well	The M L D is 0 03 mg
		237	310	0 03 „ „	Died	
		238	300	0 04 „ „	„	

noticed especially where the serum-venom mixture was in the neighbourhood of neutrality In the present writer's hands, using pigeons, such a phenomenon was never observed Even where the amount of venom injected varied by no more than 0 02 mg death either occurred almost at once or symptoms were entirely absent Where, however, the neutralizing point of the serum was closely approached it was noted that the birds might or might not die and a repeated test with the same dose of venom sometimes reversed the previous result, but still, the order of events was the same and the animals either died at once or showed no symptoms

The constancy with which the venom acted in this way made it possible to carry out and establish the correctness of the venom control before the main experiment was started This was a great saving of time because if through any experimental error or variation in the venom the M L D reading differed widely from the expected figure the test could be at once repeated and the solutions discarded, if necessary, without wasting time and animals in putting through a series of experiments with unsatisfactory venoms, as occasionally happened with the cobra venom experiments in which the controls are only readable after 24 hours Furthermore, as noted above, irregular results tended to appear as the neutralizing point of the serum was approached, and these, as will be seen later, were particularly apt to occur in the *in vivo* experiments In such cases a doubtful result could be at once checked by repeating the same dose with a fresh animal

The neutralization of daboia venom *in vivo*

The object of these tests was to ascertain the neutralizing value of the sera when the serum and venom came into contact only in the blood stream and were not previously mixed in a test-tube As pointed out by Acton and Knowles (1913) this is the condition which obtains in cases of snake-bite and the results given by the *in vitro* tests are likely to indicate a much greater potency of the serum than is the case in practice

In carrying out similar tests with cobra venom in the course of this investigation the technique was as follows The solution of venom was injected intramuscularly, ten minutes later the serum was given intravenously into the wing vein Thus it was possible to imitate very closely what actually happens in a case of snake-bite With this method, even though the results were less regular, there was surprisingly little difference in the readings from those obtained by the *in vitro* tests

Unfortunately it is not possible to employ a similar technique when working with daboia venom Although it is true that an intramuscular injection of this venom can usually be given—unless in overwhelming dose—without causing immediate death of the animal, and can therefore be followed after a short period by serum intravenously, the uncertainty of absorption of the venom and the impossibility

of determining accurately the M L D by intramuscular injection precludes the use of this method for standardizing purposes Purely for standardizing the serum, therefore, and not, let it be emphasized, in order to estimate its therapeutic value, against daboia venom *in vivo*, it is necessary that all injections, both of serum and of venom, should be intravascular The fact that sudden death follows the intravascular injection of a lethal dose of this venom forbids the injection of the serum after—even immediately after—that of the venom in these experiments Lamb (1903), recognizing this, reversed the order of injection and, using rabbits, gave the serum into one ear vein and followed this 5 minutes later by the venom into the other ear vein The writer who at this time had not come across this isolated experiment of Lamb's used, however, essentially the same technique, except that the venom was injected immediately after the serum and pigeons were the animals employed

The results of these experiments are given in Table IV

One or two interesting points are to be extracted from this table In the first place it will be seen that the neutralizing power of all the sera is of a much lower order than that exhibited *in vitro* It must be recognized, however, that the test is a very severe one in that doses up to more than 10 minimum lethal doses of venom, of which 1 M L D is sufficient to cause immediate death, are thrown direct into the blood stream It is possible that better results would have been achieved if more time had been given for the serum to circulate

Nevertheless, in spite of the severity of the test, the relatively fresh Kasauli sera exhibited a definite anti-toxic value and showed a marked difference from the sera, such as 'Serum A V', which evidently contained little or no daboia antitoxin

Secondly it will be seen that the results are much less regular than those obtained by *in vitro* tests and that irregularities are very apt to occur near the neutralizing point It is possible that these irregularities are partly due to the short time elapsing between the injections of serum and venom and that they might have been minimized had the serum been given more time to circulate Their concentration round the point of neutralization suggests, however, another more likely cause If one remembers that in 1 c c of venom solution there is often a quantity of venom equal to more than 10 M L D it is easy to understand that a very small error in the amount of solution injected might make a difference of almost 1 M L D more or less, whose effect would obviously be accentuated as the neutralizing point is approached This explanation seems the more probable from the fact that repetition of the same dose in a fresh animal frequently reversed the previous result

In all these experiments, as in the *in vitro* tests, the death of the animals either occurred almost immediately or else no symptoms were observed

TABLE IV

Neutralizing power of various antivenomous sera against daboia venom in vivo *Pigeons*

Serum injected first, intravenously, into one wing vein and followed immediately by the injection of venom solution (1 c c containing the test dose) into the other wing vein Results read after 24 hours

Venom control doses injected intravenously

Date	Serum and number	Pigeon number	Weight in grammes	Test dose	Results	Remarks
Method (a), using varying quantities of serum and a constant dose of venom						
18–10–1923	C R I 480	173	300	0 5 c c serum followed by 0 5 mg venom	Died	
		174	290	1 0 „ „ „ „ 0 5 „ „	„	0 47 mg daboia venom is neutralized by 1 5 c c serum or between this quantity and 1 1 c c
		175	280	1 5 „ „ „ „ 0 5 „ „	Alive and well	
		176	320	2 0 „ „ „ „ 0 5 „ „	„ „ „	1 c c neutralizes between 0 31 and 0 42 mg daboia venom
		177	300	2 5 „ „ „ „ 0 5 „ „	„ „ „	
18–10–1923	Daboia venom control	178	300	0 02 mg venom	Alive and well	
		179	300	0 03 „ „	Died	The M L D is 0 03 mg
		180	310	0 04 „ „	„	
Method (b), using a constant quantity of serum and varying doses of venom						
25–10–1923	C R I 481	221	290	1 c c serum followed by 0 15 mg venom	All alive and well	1 c c serum neutralizes at least 0 42 mg daboia venom (upper limit not reached by the experiment)
		222	280	1 „ „ „ „ 0 20 „ „		
		223	280	1 „ „ „ „ 0 25 „ „		
		224	250	1 „ „ „ „ 0 30 „ „		
		225	300	1 „ „ „ „ 0 35 „ „		
		226	300	1 „ „ „ „ 0 40 „ „		
		227	280	1 „ „ „ „ 0 45 „ „		

25-10-1923	C R I 421	228	280	1 c c serum followed by 0 10 mg venom	All alive and well	1 c c serum neutralizes at least 0 12 mg daboia venom (upper limit not reached by the experiment)
		229	280	1 „ „ „ „ 0 15 „ „		
		230	300	1 „ „ „ 0 20 „ „		
		231	290	1 „ „ „ 0 25 „ „		
		232	300	1 „ „ „ „ 0 30 „ „		
		233	300	1 „ „ „ 0 35 „		
		234	350	1 „ „ „ 0 10 „		
		235	300	1 „ „ 0 15 „ „		
25-10-1923	Daboia venom control	236	300	0 02 mg venom	Alive and well	The M L D is 0 03 mg
		237	310	0 03 „ „	Died	
		238	300	0 04 „ „		
6-11-1923	'Serum A V' Nos 9/12, 13, 14 pooled	299	250	1 c c serum followed by 0 03 mg venom	Died	* Nos 300 and 301 pigeons were repetitions of No 299 * Nos 303 and 304 pigeons were repetitions of No 302 * No 306 pigeon was a repetition of No 305 * No 308 pigeon was a repetition of No 307 One c c of the serum appears to neutralize only a minimal amount of daboia venom and prevents death from 1 M L D only It may, however, neutralize 0 01 mg
		300*	320	1 „ „ „ „ 0 03	Alive and well	
		301*	280	1 „ „ „ 0 03 „	„ „	
		302	300	1 „ „ „ „ 0 04 „ „	Died	
		303*	280	1 „ „ „ „ „ 0 04 „ „	Alive and well	
		304*	300	1 „ „ „ „ „ 0 04 „ „	Died	
		305	290	1 „ „ „ „ „ 0 05 „ „	„	
		306*	280	1 „ „ „ „ „ 0 05 „ „	„	
		307	280	1 „ „ „ „ „ 0 06 „ „	„	
		308*	280	1 „ „ „ „ „ 0 06 „ „	„	

TABLE IV—*concld*

Date	Serum and number	Pigeon number	Weight in grammes	Test dose	Results	Remarks
Method (b), *using a constant quantity of serum and varying doses of venom*—contd						
6-11-1923	C R I 481	309	250	1 c c serum followed by 0 40 mg venom	Alive and well	
		310*	290	1 „ „ „ „ 0 40 „ „	„ „ „	* No 310 pigeon was a repetition of No 309
		311	300	1 „ „ „ „ 0 43 „ „	Died	
		312*	290	1 „ „ „ „ 0 43 „ „	„	* No 312 pigeon was a repetition of No 311
		313	300	1 „ „ „ „ 0 46 „ „	„	* Nos 314, 315 and 316 pigeons were repetitions of No 313
		314*	300	1 „ „ „ „ 0 46 „ „	Alive and well	
		315*	300	1 „ „ „ „ 0 46 „ „	„ „ „	* No 318 pigeon was a repetition of No 317
		316*	250	1 „ „ „ „ 0 46 „ „	Died	* No 320 pigeon was a repetition of No 319
		317	310	1 „ „ „ „ 0 49 „ „	Alive and well	
		318*	300	1 „ „ „ „ 0 49 „ „	Died	1 c c of the serum certainly neutralizes 0 37 mg daboia venom It may neutralize 0 43–0 46 mg
		319	300	1 „ „ „ „ 0 52 „ „	„	
		320*	300	1 „ „ „ „ 0 52 „ „	„	
		321	310	1 c c serum followed by 0 06 mg venom	Alive and well	
		322	290	1 „ „ „ „ 0 07 „ „	„ „ „	
		323	290	1 „ „ „ „ 0 08 „ „	„ „ „	
		324	290	1 „ „ „ „ 0 09 „ „	„ „ „	
		325	280	1 „ „ „ „ 0 10 „ „	„ „ „	The clear supernatant fluid serum was used, without shaking up the deposit
		326	290	1 „ „ „ „ 0 13 „ „	„ „ „	

6-11-1923	C R I 252	327	290	1 „ „ „ „ 0 16 „ „	„ „ „	1 c c serum neutralizes at least 0 28 mg daboia venom and may neutralize 0 31 mg
		328	280	1 „ „ „ „ 0 19 „ „	„ „ „	
		329	320	1 „ „ „ „ 0 22 „ „	„ „ „	The intermediate dose 0 34 mg was omitted in error
		330	300	1 „ „ „ „ 0 25 „ „	„ „ „	
		331	320	1 „ „ „ „ 0 28 „ „	„ „ „	This serum was time expired
		332	290	1 „ „ „ „ 0 31 „ „	„ „ „	
		333	250	1 „ „ „ „ 0 37 „ „	Died	
		334	260	1 „ „ „ „ 0 40 „ „	„	
6-11-1923	C R I 394	335	300	1 c c serum followed by 0 31 mg venom	Alive and well	* No 339 pigeon was a repetition of No 338
		336	310	1 „ „ „ „ 0 34 „ „	„ „	
		337	250	1 „ „ „ „ 0 37 „ „	„ „ „	
		338	320	1 „ „ „ „ 0 40 „	Died	* Nos 341 and 342 pigeons were repetitions of No 340
		339*	250	1 „ „ „ „ 0 40 „ „	Alive and well	
		340	300	1 „ „ „ „ 0 43 „ „	„ „ „	1 c c of the serum certainly neutralizes 0 34 mg daboia venom and most probably neutralizes 0 40 mg
		341*	250	1 „ „ „ „ 0 43 „ „	„ „ „	
		342*	260	1 „ „ „ „ 0 43 „ „	„ „ „	
		343	250	1 „ „ „ „ 0 46 „ „	Died	
		344	250	1 „ „ „ „ 0 49 „ „	„	
6-11-1923	Daboia venom control	345	310	0 02 mg venom	Alive and well	The M L D is 0 03 mg
		346	270	0 03 „ „	Died	
		347	300	0 04 „ „	„	

Discussion

The experiments described in this paper were carried out primarily with a view to comparing the neutralizing value of an unknown serum with that of a serum which from its mode of preparation was assumed to possess some anti-toxic effect on daboia venom From this point of view the tests were completely successful and the difference between the two types of sera is quite definite

As a secondary consideration it was desired to ascertain whether a comparatively simple biological test, on the lines used with cobra venom as a routine, could also be reliably employed to standardize a serum specifically prepared against Russell's viper venom The results indicate that the methods used here can be applied for this purpose with little greater difficulty or liability to error than is experienced in the routine standardizing test used at Kasauli, provided certain precautions are observed

The results of such experiments as these are, of course, not directly translatable into an estimate of the effect that may be expected from therapeutic use of the serum in cases of Russell's viper bites, and it is quite impossible to say whether the figures obtained by the *in vitro* method or *in vivo* come nearer to the neutralizing value of the serum in such cases Purely as a standard, however, with a view to preventing the issue of serum of poor value these two methods give satisfactory results

As to the choice between standardization *in vitro* or *in vivo*, the technical difficulties of the latter method and its greater tendency to lead to irregular results suggest that *in vitro* standardization is more suitable for routine work The *in vivo* test, unlike the corresponding test with cobra venom, is perhaps more of academic interest as showing what the serum can do even under such severe conditions of test The conditions are such that reproduction of them in cases of snake-bite must occur excessively rarely, and if they did, no subsequent serum treatment could possibly be applied quickly enough to save life

Whatever method is used, however, for standardization purposes one thing is clear, namely that intravascular injection is essential Only in this way can the M L D be accurately determined and accurate determination of the M L D is the basis of any biological standardization method The M L D should be regarded as the smallest dose which kills by intravascular clotting, and this amount should, of course, be deducted from the total dose of venom, in calculating the quantity neutralized by the serum

It is tentatively suggested that a serum of which 1 c c will neutralize *in vitro* 1 5 mg of daboia venom is suitable for issue

The choice of animal appears to the writer to be important Various workers have used different animals , mice, rats, guinea-pigs, monkeys and most commonly rabbits and pigeons Of all these the rabbit and the pigeon are by far the easiest to deal with from the technical point of view when it is a question of intravascular

injections As regards the choice between the rabbit and the pigeon, in the writer's opinion the pigeon is more suitable

There is not much difference in the ease with which intravenous injections can be given to these two animals though on the whole, the writer, after very considerable experience with both animals considers the pigeon easier, owing to the large and prominent wing veins

Pigeons are, however, easier to handle and much cheaper, and, what is of great importance, remarkably uniform in weight when adult, so that there is no need to calculate individual doses on any dose for weight principle The writer has carried out over several years a very large number of animal experiments involving the use of hundreds of pigeons and has never had any difficulty in selecting sufficient birds weighing from 280 to 310 grm With birds of this weight the M L D both of cobra and daboia venom is astonishingly constant provided of course that the same sample of venom is used

As mentioned earlier both Lamb and Acton and Knowles, when carrying out neutralizing tests with rabbits observed that some of the animals showed delayed death or survived with marked symptoms This was never the experience in the writer's experiments Whether this was due to the use of pigeons instead of rabbits or because he was lucky in finding a good sample of venom—for venoms vary as will be seen later—is not known It is, however, at least possible that the choice of animal was responsible, not perhaps because animals of different species act differently—or not markedly differently—from one another to the effects of daboia venom, but rather dependent on the site of injection and its proximity to the heart and great vessels In the pigeon the site of injection is the large wing vein, leading almost directly to the right heart, as opposed to the relatively long distance from the heart and the small size of the ear vein of the rabbit

However this may be, the fact remains that injection of a fatal dose of venom, whether alone or in excess of that neutralized in a mixture of serum and venom, never failed to kill suddenly, while a fractionally smaller but non-fatal dose produced no observable effect

A further indication that all animals do not react alike to daboia venom, again probably owing to the distance of the site of injection from the heart, is given by an observation of Lamb and Hanna (1903) on monkeys In these animals they found that intravenous injection into the cephalic vein of the arm of a dose of daboia venom just insufficient to cause death by intravascular clotting, or the *slow* injection of a dose just sufficient, if given rapidly, to kill by intravascular clotting, produced transient symptoms of greater or less severity, and that the subsequent injection into the blood stream of a much larger dose, which would normally be rapidly fatal, failed to produce intravascular thrombosis but was either without effect or killed by chronic intoxication In their experiments with rabbits described in the same paper this effect did not appear They say 'Further it would appear that even

when a dose (of daboia venom) which just fails to cause death from intravascular thrombosis is given intravenously, the animal presents none at all or only very slight symptoms'

In addition to the desirability of choosing a suitable animal it is necessary to select a suitable sample of venom That daboia venoms vary in their effect appears to have been noticed by Lamb In nearly all his work with this venom he refers to the M L D as being the minimum amount which caused death by intravascular thrombosis when injected intravenously This, in the rabbit, was about 0 2 mg per kilo In one experiment, however (Lamb, 1903), he used as his test dose 0 5 mg per kilo, which, he says, was 'an amount which as regards this sample of venom did not cause intravascular thrombosis when injected intravenously' This amount *of venom, however, when injected intravenously in a mixture with an inert serum* killed the animals after a more or less prolonged period This sample, therefore, was presumably deficient in its blood coagulant action

Some subsequent work of the present writer also showed how much the venom may vary A number of samples were met with in which it was not possible to determine the M L D by making use of the blood coagulant effect by intravenous injection

TABLE V

Daboia venom (Sample A) *Determination of the minimum lethal dose by intravenous injection Pigeons*

One c c of venom solution containing various doses, injected into the wing vein Results read after 24 hours

Date of test	Pigeon number	Weight in grammes	Test dose	Results	REMARKS
14-2-1924	77	300	0 01 mg venom	Alive and well	
	78	310	0 02 „ „	„ „ „	
	79	310	0 03 „ „	„ „ „	
	80	310	0 04 „ „	Died	No 80 died in 10 minutes
	81	300	0 05 „ „	„	No 81 died in 5 minutes
15-2-1924	88	300	0 02 mg venom	Alive and well	
	89	300	0 03 „ „	Died	
	90	310	0 04 „ „	„	
	91	310	0 05 „ „	Alive and well	

TABLE V—*concld*

Date of test	Pigeon number	Weight in grammes	Test dose	Results	Remarks
15-2-1924	92	300	0·02 mg venom	Alive and well	
	93	300	0·03 „ „	„ „ „	
	94	300	0·04 „ „	„ „ „	
	95	300	0·05 „	Died	
15-2-1924	96	290	0·02 mg venom	Alive and well	
	97	300	0·03 „ „	Died	
	98	300	0·04 „ „	„	
	99	300	0·05 „ „	Alive and well	

Table V shows how variable the results may be with certain samples of venom, some of which are quite useless for standardization purposes It is probable, therefore, that this venom varies considerably in its constitution, and this aspect of the problem requires further investigation

CONCLUSIONS

(1) Methods of standardizing anti-daboia serum, by means of its neutralizing power *in vitro* and *in vivo* have been described and the results of experiments given

(2) The *in vitro* method, by reason of the ease with which it can be carried out and the regularity of the results obtained, appears to be more suitable for routine work and is considered to provide a reliable method of standardizing a serum against daboia venom

(3) The results of these tests are not to be interpreted as directly related to the quantity of daboia venom which will be effectively neutralized by serum used therapeutically in cases of snake-bite, but only as indicating a standard which may be used to prevent the issue of serum of poor value

(4) It is suggested that anti-daboia serum of such a potency that 1 c c will neutralize *in vitro* at least 1 5 mg daboia venom is suitable for issue

(5) For any biological method of standardizing antivenin against daboia venom all injections, both of serum, or venom, or mixtures of both, should be given directly into the blood stream

(6) Evidence is put forward that the choice of animal for use in such standardizing tests is important, the suitability of the animal probably increasing

mosquitoes were dissected and they showed many young filariæ in the body cavity and thoracic muscles During the subsequent period also, the mortality among the mosquitoes was high, although not as heavy as that observed on the first day

In 26 specimens out of 34 dissected on the 4th to 11th day, filariæ were observed in different stages of development The rate of development of the filariæ was found to be similar to that observed in *Culex fatigans* which was used as control Dissections carried out on the 4th, 6th and 7th days showed normal development of the filariæ In one specimen dissected on the 11th day after feed, mature larvæ were seen in the thorax

Fresh-water A subpictus

Out of 29 mosquitoes dissected, 21 took the infection and showed developmental phases of *W bancrofti* The rate of development of the worm larvæ was normal and did not differ from salt-water *A subpictus* As with the latter race, there was heavy mortality among mosquitoes that took the infective feed Dissections on the 4th and 6th days showed filariæ in their respective stages of development On the 10th day, the stage prior to the mature larva stage was observed Owing to heavy mortality among the mosquitoes, dissections were not delayed long enough for the mature larva stage to be observed It seems likely that the filariæ would complete their larval development in due course if the infected mosquitoes survive for that period

Anopheles subpictus is thus seen to be susceptible to infection with *W bancrofti* and the larvæ are able to complete their development in this species There appears to be a severe reaction in the host consequent to the infection and it suffers a high mortality, especially on the day following the day of feed The salt-water race of *A subpictus* did not differ materially from the fresh-water form in its susceptibility to infection

(4) ANOPHELES LUDLOWII *var* SUNDAICA

Several infection experiments were carried out with this species and altogether 41 mosquitoes survived for the dissections Out of the 41 mosquitoes dissected, 28 were positive for filariæ The number of filariæ observed in the mosquitoes was fairly high , in several specimens, 10 to 18 filariæ were seen in each The rate of development was quite rapid On the 3rd day after feed, sausage forms were found in good numbers On the 5th day, elongated forms were common On the 10th day, mature larvæ were seen in the thorax In one specimen, six mature larvæ and several immature ones were seen on the 10th day

These results show that *A ludlowii* var *sundaica* is a favourable host for *W bancrofti* On a previous occasion while dissecting wild specimens of this species collected from the vicinity of Budge Budge (24-Perganas District) for infection

with malaria parasites, one of us (M O T I) observed a natural filarial infection in two specimens out of a total of 711 The mature larvæ found in these two specimens were measured and they conformed in size and in appearance to mature larvæ of *W bancrofti*

(5) ANOPHELES STEPHENSI

Ten batches of *Anopheles stephensi* were fed on filarial cases Out of a total of 39 mosquitoes dissected in this series on different days following the day of infective feed, 36 were found to be infected The experimental infection rate in this species is very high The number of filariæ per infected mosquito was also high In most of the infected specimens, there were more than 10 filariæ and in several, as many as 30 were seen *Anopheles stephensi* is very susceptible to infection and it appears to be a favourable intermediate host of *W bancrofti* The rate of development of the filariæ in *A stephensi* was even more rapid than in *Culex fatigans* which was run as control As early as on the 10th day after the infective feed, several mature actively moving larvæ were observed in the thorax and abdomen of experimentally infected *A stephensi* In the case of *Culex fatigans* which were experimentally infected during the same period as the present observations, it was only on the 11th day that mature filaria larvæ were observed in the mosquito and the filariæ were not observed in the head and proboscis earlier than the 12th day In experimentally infected *A stephensi*, many mature larvæ were observed in the thorax and abdomen on the 10th day and a larger number reached maturity on the 11th day Mature larvæ were observed in the head and proboscis of *A stephensi* even on the 11th day

The results show that *W bancrofti* develops very rapidly in *A stephensi* and judged from the high infection rate and the heavy infestation observed under experimental conditions, this species is an efficient intermediate host of *W bancrofti*

(6) ANOPHELES FULIGINOSUS

Twelve batches of *A fuliginosus* mosquitoes were fed on filarial cases Altogether, 43 specimens of the fed mosquitoes were dissected from the 7th to 15th day after feed The infection rate was found to be high, 30 out of the 43 dissected had filaria larvæ The filariæ developed very well in this species and the rate of development was as rapid as that observed in *A stephensi* Mature larvæ were observed in the thorax and abdomen on the 10th day after feed and on the 11th day, active mature larvæ were seen in the head and labium

(7) ANOPHELES PSEUDOJAMESI

Four batches of *A pseudojamesi* were fed on filarial cases and 15 were dissected on the 6th to 10th day after the infective feed Six out of the 15 were found infected, and the number of filariæ found in the mosquitoes was small, being between 1 and 4

The rate of development was normal, on the 10th day, the filariæ in the thorax were in the stage prior to the final mature stage The number of observations carried out with this species is small, the present results show that *A pseudojamesi* is not an unsuitable host for *W bancrofti*

(8) Anopheles varuna

Three batches of mosquitoes were given infective feeds and of these 19 mosquitoes were dissected on the 5th to 10th day after their respective infective feeds A high infection rate was observed with this species, 16 out of the 19 mosquitoes dissected were found to be infected The rate of development of the filariæ in *A varuna* was observed to be similar to that observed in *Culex fatigans* On the 10th day after feed, the stage immediately prior to the mature stage was observed From 3 to 10 filariæ were observed in the infected mosquitoes

(9) Anopheles pallidus

Three batches of *Anopheles pallidus* were fed on filarial cases and 42 mosquitoes were dissected on the 4th to 11th day A fairly high infection rate was observed in *A pallidus*, 28 specimens out of 42 examined were found infected The number of filariæ found in the infected mosquitoes was also very high In several specimens, as many as 30 to 60 filariæ were observed The rate of development of the filariæ was as in *Culex fatigans*, on the 11th day after feed, mature larvæ were observed actively moving about in the thorax and abdomen

(10) Anopheles philippinensis

Only a small number of observations were made with *A philippinensis* Out of three mosquitoes that were dissected on the 4th day, all of them were found infected The number of filariæ found in each mosquito was observed to be between 21 to 23 Owing to lack of bred-out specimens, a larger number of observations were not carried out

Some specimens of *A philippinensis* caught as adults from houses in a filarial area in Nadia District were dissected and natural filarial infections were observed in two out of ten specimens examined Both of these positive specimens had mature filaria larvæ which were similar in appearance as well as in size to mature larvæ of *W bancrofti*

(11) Culex fatigans

In conjunction with many of the infection experiments discussed herein *Culex fatigans* mosquitoes were fed on the same cases for purposes of comparison Altogether 24 batches of mosquitoes were fed on filarial cases and 212 mosquitoes were dissected Out of these, 152 mosquitoes were found to be infected The number of filariæ in these mosquitoes was generally between 8 to 10, in some

specimens, a larger number of filariæ (15 to 20) were observed In this series of observations the mature larval stage of the filaria was observed in the thorax and abdomen of *Culex fatigans* on the 11th day The larvæ were not observed in the head and proboscis earlier than the 12th day

(12) CULEX VISHNUI

Only four specimens of *Culex vishnui* were available for dissection after the infective feeds Of these three were negative while one which was dissected on the 4th day showed two young sausage shaped filariæ The rate of development seemed to be normal

(13) ÆDES (STEGOMYIA) AEGYPTI

Twelve batches of *Ædes* (*Stegomyia*) *ægypti* were fed on filarial cases and the mosquitoes were subsequently dissected or serially sectioned Out of 50 mosquitoes thus examined, only three showed filariæ In one specimen, two dead filariæ of the stage analogous to the 2nd day stage in *Culex fatigans* were observed on the 7th day after feed In the second positive specimen, two small forms similar to the 2nd day stage were seen in the thoracic muscles on the 8th day after feed In the third specimen, four minute filariæ, also of the 2nd day stage, were observed on the 13th day after feed In 47 out of 50 mosquitoes examined either by dissections or by serial sections, no trace of the filariæ was noticeable

Dissections and sections of *Ædes ægypti* carried out on the day following the date of feed showed numerous microfilariæ within the stomach and it was observed that many of the microfilariæ had cast the sheath After this stage the fate of the filariæ is not definitely known A small number of these probably manage to enter the body cavity of the mosquito, but the majority of them do not succeed in doing so and are probably voided with the fæces Even such of the filariæ that enter the body cavity and effect a lodgment in the thoracic muscles do not seem to have any chance of completing their larval development Even on the 8th day after feed, the filariæ observed in the mosquito were still very small and undeveloped The same condition was observed even on the 13th day after the infective feed, and as such the filariæ have very little chance of completing their larval development The results show that *Ædes* (*Stegomyia*) *ægypti* is not a favourable intermediate host for *W bancrofti*, as most of the microfilariæ fail to effect an entry into the body cavity of the mosquito, and the development of the few that may actually effect an entry is suppressed

(14) ÆDES (STEGOMYIA) VITTATUS

Experimental infection of this species was attempted by one of the authors (S S R) while working in Cuttack in October 1921 In this series 27 mosquitoes were dissected 9 to 14 days after the infective feed and the results were entirely

negative *Ædes* (*Stegomyia*) *vittatus* was observed to be unfavourable for the development of *W bancrofti*

(15) Armigeres obturbans

Eleven batches of *Armigeres obturbans* were fed on filarial cases and 98 mosquitoes were dissected or sectioned The results have been totally negative In dissections carried out on the day following the day of feed, the authors observed microfilariæ without sheaths in the stomach contents, and in two instances, one to two microfilariæ were observed in the body cavity of the mosquito In no instance were any filariæ seen in the thoracic muscles of the mosquito, either in serial sections or in dissections The results show that *Armigeres obturbans* is unsuitable as an intermediate host of *W bancrofti*

(16) Tæniorhynchus (Mansonioides) annuliferus

Six mosquitoes were fed on filarial cases and dissected on the 5th to 6th day They were negative for filariæ The number of observations with this species is small

Summary

Several species of Indian mosquitoes have been found to be efficient intermediate hosts of *W bancrofti* under experimental conditions Other species were entirely refractory to the infection Out of sixteen species experimented on in this series *Armigeres obturbans* and *Ædes* (*Stegomyia*) *vittatus* were observed to be proof against infection with *W bancrofti* *Tæniorhynchus* (*Mansonioides*) *annuliferus* also gave negative results, but as only six observations were made with this species, the negative results are not of much value *Ædes* (*Stegomyia*) *ægypti* was found to be unfavourable as an intermediate host of *W bancrofti* Most of the specimens of this species did not take the infection and in the few that took the infection, the filariæ were arrested in the very early stages of development and they did not grow any further In *Culex vishnui*, experimental infection was observed in a single specimen out of four examined, a larger number of observations should be made to ascertain its susceptibility and to see if the filariæ would complete their larval development in this species *Anopheles hyrcanus* var *nigerrimus* and *A barbirostris* are both susceptible to infection, filaria larvæ completed their development in these two species, but the development of a large proportion of the filariæ is greatly suppressed and they may not reach maturity at all In the three species *A subpictus*, *A pseudojamesi* and *A varuna*, the larvæ of *W bancrofti* were observed to develop normally as in *Culex fatigans*, but the filariæ found in the mosquitoes were comparatively few in number There was no marked difference in the susceptibility of the fresh-water and salt-water races of *A subpictus* *Culex fatigans*, *Anopheles philippinensis*, *A pallidus*, *A fuliginosus*, *A stephensi* and

TABLE

Serial number	Species of mosquito	Number of specimens examined	Number found with filariæ	Number of filariæ found in one specimen			Time taken for development of mature larva
				Maximum	Minimum	Average	
1	*Anopheles hyrcanus* var *nigerrimus*	27	15	32	1	6 7	12 days
2	*Anopheles barbirostris*	10	4	4	3	3 5	15 days
3	*Anopheles subpictus*—						
	(*a*) Salt water race	34	26	20	5	8 5	11 days
	(*b*) Fresh water race	29	21	15	1	5 1	
4	*Anopheles ludlowii* var *sundaica*	41	28	18	1	7 0	10 days
5	*Anopheles stephensi*	39	36	30	2	12 2	10 days
6	*Anopheles fuliginosus*	43	30	12	1	4 8	10 days
7	*Anopheles pseudojamesi*	15	6	5	1	2 5	
8	*Anopheles varuna*	19	16	18	2	6 5	
9	*Anopheles pallidus*	42	28	55	1	14 9	11 days
10	*Anopheles philippinensis*	3	3	23	21	22 3	
11	*Culex fatigans*	212	152	20	2	7 2	11 days
12	*Culex vishnui*	4	1	2		2 0	
13	*Ædes* (*Stegomyia*) *ægypti*	30	3	4	2	2 7	Not likely to mature
14	*Ædes* (*Stegomyia*) *vittatus*	27	0				
15	*Armigeres obturbans*	98	0				
16	*Tæniorhynchus* (*Mansonioides*) *annuliferus*	6	0				

A ludlowi var *sundaica* were found to be efficient intermediate hosts of *W bancrofti* With the exception of *A philippinensis*, in which only a small number of observations was made, in all the other species, *W bancrofti* was observed to complete the larval development rapidly and in fair numbers In *Culex fatigans* and *Anopheles pallidus*, the time taken for the larva to reach maturity was observed to be 11 days In *Anopheles fuliginosus*, *A stephensi* and *A ludlowi* mature larvæ were observed as early as the 10th after infective feed

In addition to these experimental infections, natural infections were observed in specimens of *A ludlowi*, *A philippinensis* and *Culex fatigans* caught in human habitations

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NOTES ON SPONTANEOUSLY AGGLUTINATING STRAINS OF *V CHOLERÆ* BOTH NATURAL AND ARTIFICIALLY PRODUCED

BY

AMAR NATH GOYLE, M B , PH D ,

AND

P N SEN GUPTA, M SC

(*From the Pasteur Institute of Burma, Rangoon*)

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THE question whether there are several serological types of *V choleræ* has been studied by numerous workers and in spite of certain observations to the contrary it is fairly well established that in the stools of cases of cholera vibrios are found which may exhibit marked differences in their agglutinating properties It was considered that the recent investigations into the dissociation of micro-organisms might throw some light on this subject Those interested in the literature of this subject are referred to the comprehensive monograph on 'Microbic Dissociation' by Hadley (1927) and to an article by Arkwright (1930) on the same subject in 'A System of Bacteriology' (*Medical Research Council*)

The observations presented below are of some interest inasmuch as variants have been obtained from cultures of *V choleræ* in the laboratory which react serologically with cholera organisms isolated from actual cases of cholera It may be added that these variants while differing from the true *V choleræ* in their agglutinability in peptone water medium are identical with it in their growth characters on agar

SOURCE OF THE CHOLERA STRAINS INVESTIGATED

1 *V choleræ* 67S was isolated from a case of clinical cholera in the Contagious Diseases Hospital, Rangoon No clinical history is available

2 *V choleræ* 547S , Contagious Diseases Hospital, Rangoon Date of onset of symptoms of cholera, 18-5-30 Condition on admission on 21-5-30 Emaciated, passing frequent watery stools with scanty urine Cholera vibrio isolated on 22-5 30 Died on 28-5-30 Very weak phage obtained from the stool on 22-5-30

3 *V cholerae* CD R was isolated from a case of cholera in 1929 in the Contagious Diseases Hospital, Rangoon No clinical history is available

4 *V cholerae* 609R was isolated by Dr Asheshov from a cholera case in Patna Hospital on 18-7-29 Dr Asheshov states that the colonies present on the first plating were of the rough type, but on plating we found no colony with rough appearance

5 *V cholerae* P I B S This was originally obtained from the National Collection of Type Cultures maintained at the Lister Institute of Preventive Medicine, London There is, however, no record to indicate its identity with any of the strains now kept there

6 *V cholerae* 67R , 547R and P I B R were obtained from the corresponding S strains as described below

Methods of obtaining variants

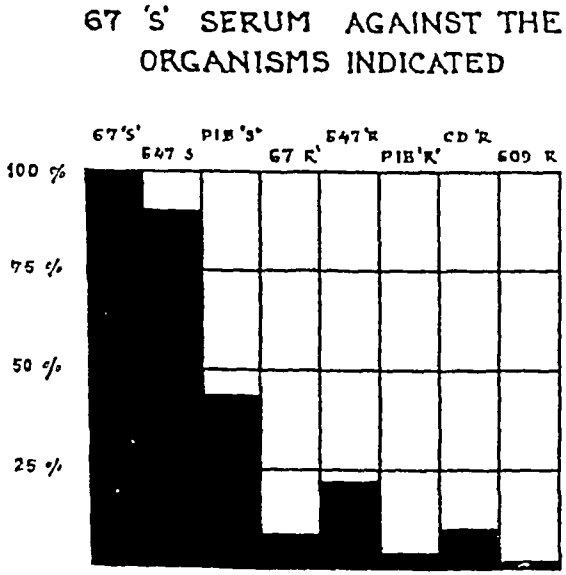
The cultures 67S , P I B S and 547S were purified by three successive platings and single colony isolation Cultures thus purified were inoculated into peptone water and left for ageing Immune serum being an active dissociating agent, the cultures were inoculated into 10 per cent 67S immune serum in peptone water Serial sub-cultures were made at weekly intervals, agar plates being spread out at each time of sub-culture No rough colony was found even after the third passage Spontaneous agglutination in broth being a characteristic of the R form, six colonies were taken from the agar plate spread from the fourth sub-culture and put into peptone water None showed agglutinated growth Colonies showing agglutinated growth began to appear in the plates spread out from the 6th and 7th sub-cultures, though none of the colonies showed rough appearance on agar Once a colony showing good agglutinated growth was obtained, it was further purified by repeated plating and colony selection until a colony was obtained which, when spread on agar, gave colonies any twelve of which when put into peptone water gave agglutinated growth The cultures thus finally obtained were tested for stability by repeated peptone water sub-cultures at 24 hours intervals for two weeks, after which no sign of reversion was observed From the peptone water cultures left for ageing plates were spread out at monthly intervals No colony showing rough appearance or agglutinated growth in peptone water was obtained even after six months

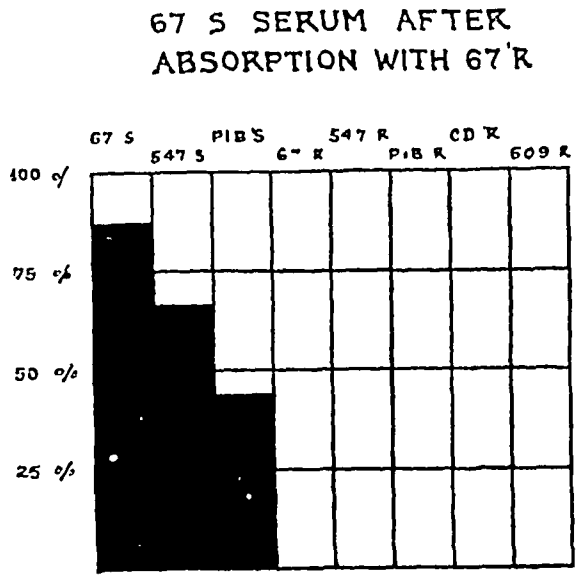
Agglutination tests

Rabbits were inoculated intravenously with 67S , 67R and CD R at weekly intervals and after five injections the agglutinating titre of the sera was as follows —

67S serum	.	1 in 9,000
67R „	.	1 in 8,000
CD R „ ..	.	1 in 15,000

The results of agglutination of the different strains with these sera are shown in Figs 1, 2 and 3 The serum made with 67S agglutinates the S forms of *V cholera* to a titre varying from 1-1,000 to 1-9,000, i e , 44 to 100 per cent On





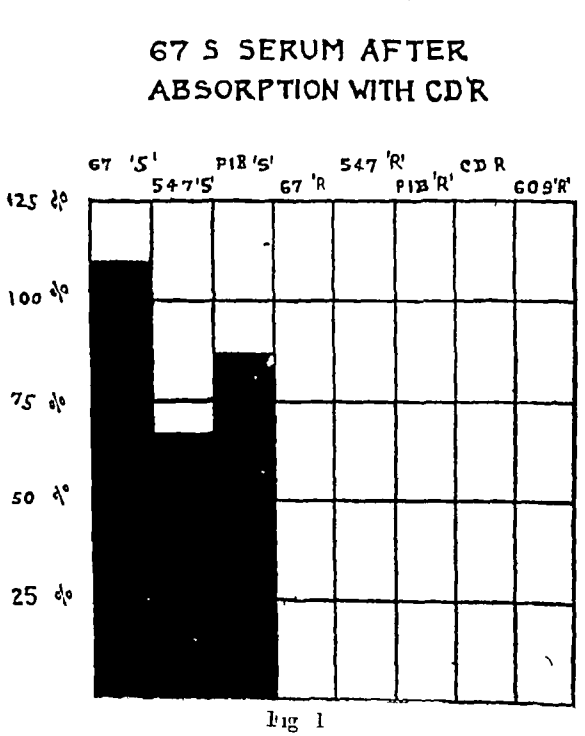


Fig 1

the other hand the same serum agglutinates the R forms to a much lower titre, namely 2 to 22 per cent The serum made with 67R reacts with the R form of the different strains to full titre (with CD R slightly beyond titre) but S forms are agglutinated only to 6 to 24 per cent of the titre (Fig 2) Results of experiments with CD R serum (Fig 3) are similar to those with 67R serum

It is thus seen that there is only slight cross-agglutination between the S and R variants

Absorption tests

The results of absorption tests are shown in Figs 1–3 These indicate that 67S serum after its contact with 67R or CD R still agglutinates to a relatively higher titre the S forms of the different strains but has lost whatever agglutinins it had possessed for the R forms of cholera vibrios When 67R *serum is absorbed* with the S form, there is complete removal of the agglutinins for S forms* but the titre for R forms is altered very little It will be noticed that the CD R serum is

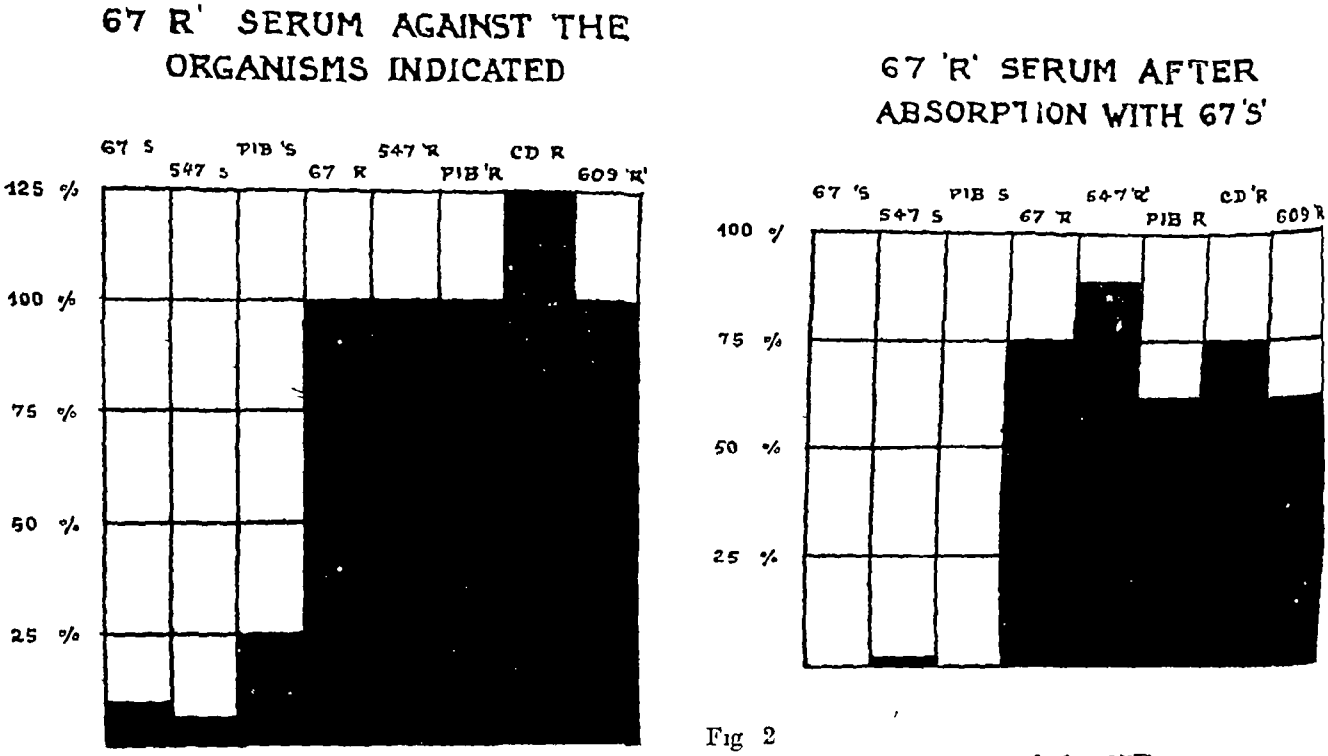


Fig 2

identical with 67R serum in its behaviour (Fig 3) Absorption of the 67R serum with CD R and vice versa leaves no trace of any agglutinins It may be noted that a serum after absorption with the homologous strain was devoid of any agglutinating power in any of the strains used These results have not been recorded in the figures for the sake of brevity

* Except in the case of 5475 which is agglutinated to 2 per cent of the titre

MISCELLANEOUS EXPERIMENTS

A *Hæmolytic action* —None of the strains under investigation showed any lytic effect on sheep's red cells For the purpose of this test we used three days old peptone water cultures of the different strains These were centrifuged and the supernatant fluid was added in different dilutions to an equal quantity of sheep's red cells in small test-tubes

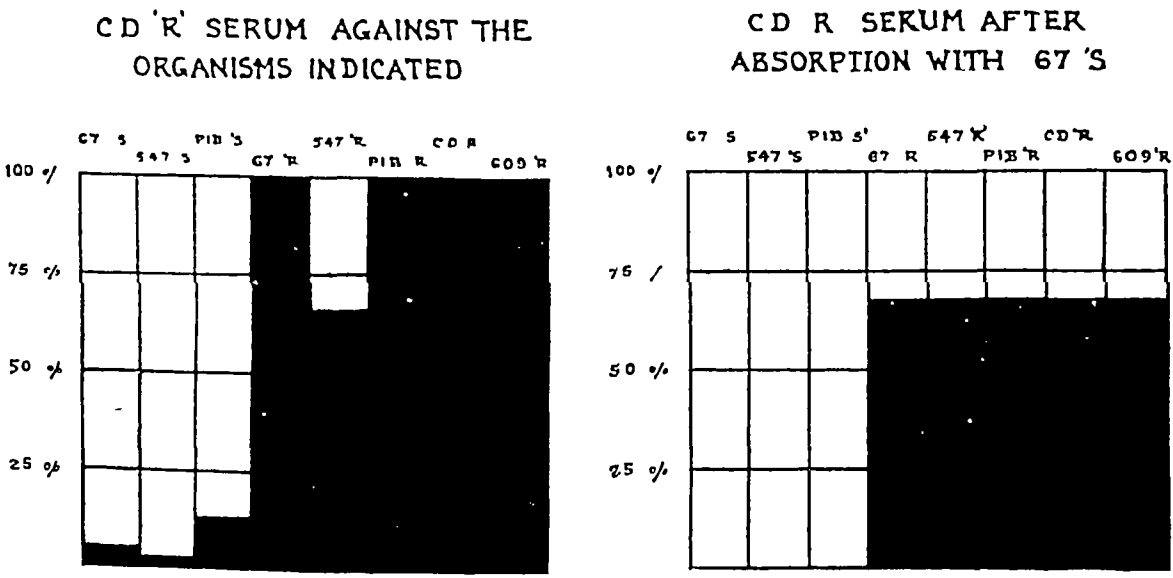


Fig 3

B *Salt agglutination* —All the eight cultures were tested for salt agglutination and the result is recorded in the following table —

STRAINS	$\frac{M}{2}$ NaCl	$\frac{M}{4}$ NaCl	$\frac{M}{8}$ NaCl	$\frac{M}{16}$ NaCl	$\frac{M}{32}$ NaCl
67S	–	–	–	–	–
PIBS	–	–	–	–	–
547S	–	–	–	–	–
67R	+++	+++	±	–	–
CD R	+++	+++	±	–	–
PIBR	+++	+++	±	–	–
609P	+++	+++	±	–	–
547R	+++	+++	±	–	–

C *Testing with Millon's reagent*—One loopful of 24 hours agar cultures of each of the eight cultures was emulsified in 2 c c of distilled water taken in clean test-tubes To each 5 drops of Millon's reagent were added In 1-2 minutes all the rough cultures began to show clumping On standing after boiling for 1-2 minutes, the rough cultures gave a red deposit, leaving a clear supernatant fluid, while the smooth cultures showed no change

D *Bio-chemical reactions*—No difference was observed between the smooth and rough cultures as regards their bio-chemical reactions All formed acid in glucose, mannite, maltose and saccharose, lactose and dulcite remaining unchanged All gave negative Voges-Proskauer reaction and positive cholera red reaction

Discussion

V choleræ is a very delicate organism and alterations in its agglutinability occur somewhat readily Modifications in agglutinability may result from the action of various factors Among recent workers Nadake (1920) and Yamanouchi (1921) observed that cholera vibrios grown in homologous serum lose their agglutinability, which is also diminished by water culture (Zlatogoroff, 1909) and the action of phage (d'Herelle and others, 1930) Vibrios isolated from cases of cholera also show marked differences in agglutinability

In the present paper we are only concerned with alterations of agglutinability associated with spontaneous agglutination Shousha (1924) obtained R forms from a hæmolytic and a non-hæmolytic strain of *V choleræ* by plating old broth cultures It may be noted that his description of these forms suggests that they are identical with the well-known rough forms of intestinal bacteria He cites Hamburger as saying that cholera vibrios when grown in immune serum show spontaneous agglutination, but on repeated sub-culture on agar this character was lost

It is not yet possible to say with certainty that the forms described in the present paper are rough though their sensitiveness to salt and comparative inagglutinability with the parent serum are in favour of the view that they are R forms It may be recalled that Goyle (1926) studied a strain of *B typhosus* (162) which was found to be smooth in cultural features and in its stability in physiological saline, but which exhibited a fair amount of the R antigen when tested serologically In other words R antigen may exist in apparently smooth cultures and its presence may be determined only by agglutination and absorption tests with heated and unheated emulsions The form of *V choleræ* we have studied is agglutinated in normal saline and this character has not been lost even after repeated sub-cultures on agar The correlation of this form with the well recognized rough forms of Arkwright though not definitely established appears probable Further the R form obtained by growing the S forms in immune serum have been shown to be serologically identical with the two strains isolated from clinical cholera stools and their agglutination with the S serum is slight It is a well-known fact that

magglutinable or less agglutinable vibrios are more commonly met with during convalescence and the subsidence of an epidemic than at its height It is probable that modifications in the agglutinability of vibrios occur in the intestine under conditions of advancing immunization and some of these at any rate are identical with the changes that occur in the vibrios when grown in immune serum In this connection it is interesting to note that Yamanouchi (1921) found the agglutinability of vibrios diminished after their introduction into the alimentary tract of a cholera immune animal but its agglutinogenic power was not diminished The advantages of a standard agglutinating serum in the diagnosis of cholera are obvious and a study of the variants produced under natural and artificial conditions in our opinion would afford considerable help in the production of such a serum

Summary

1 The experiments recorded in this paper tend to support the view that *V choleræ* produces variants under natural as well as artificial conditions

2 The form described in this paper does not produce the characteristic rough colonies of other intestinal bacteria on agar but is spontaneously agglutinable

3 The form obtained by growing the S form in immune serum has been shown to be serologically identical with the spontaneously agglutinating strains isolated from cases of clinical cholera

4 The R form whether derived artificially or naturally differs from the S form in its agglutinating absorbing and antigenic properties

5 It is suggested that some of the magglutinable strains met with in nature may be of this type, i e , ' rough ', and ' roughness ' may be revealed only on serological investigation

6 The two forms (S and R) are identical as regards fermentation of carbohydrates, formation of indol and absence of hæmolytic action

7 With the Millon's reagent the two forms react differently

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AN EXPERIMENTAL CONTRIBUTION TO THE DIAGNOSIS OF PERNICIOUS ANÆMIA

BY

K N MURTHI MB, BS,

Research Fellow in Medicine, University of Madras,

AND

P JOHN SUNDARA RAO, MA, FZS,

Department of Biology, Medical College, Madras

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While investigating the subject of pernicious anæmia at the Government General Hospital, Madras, the first author was baffled with the problem of demonstrating the toxicity of the sera of cases of pernicious anæmia Injections of the serum and whole blood from cases of pernicious anæmia have been given to the albino rats and guinea-pigs in order to make a study of the toxicity of the sera All those experiments have failed so far to give any clue for the demonstration of the toxicity Weights and temperatures have been noted and changes in blood pictures have been studied in the animals that have been experimented upon, but none of them gave any definite conclusive result It was suggested that instead of animals young seedlings might give a clue for the demonstration of the toxicity of the sera Having this suggestion in mind we looked into the literature to see if there had been any experimental work done on this subject The earliest work that has been reported was in 1922, and in 1926 by Macht in *Jour of Phar and Exper Ther*, and subsequently another contribution had been made by Macht in the *Jour of Amer Med Assoc*, September 3, 1927, Vol LXXXIX, pages 753 to 759 After this there was but little available literature on the subject In collaboration with the second author the following phytopharmacological experiments have been carried out There was a great difficulty in the selection of the seedlings for our experimental purposes *Lupinus albus* were the seeds used by the research workers in America, but we could not obtain these for our experiments Various seeds were first germinated in order to find which of them was most suitable in quickly germinating and in giving straight tap roots to allow accurate measurements of their lengths from day to day Beans, peas

and other germinating grains were found to be very defective in satisfying the above needs The only seeds that nearly approximated to our requirements are those of *Vigna catiang* (Plate I, fig 1) The plant belongs to the Natural Order Leguminoseæ These seeds are obtainable anywhere in the Indian market being freely used as an article of food by the Indians The seeds are of small size and cylindrical shape

We tried to germinate the seeds in sand and then transfer them to the apparatus containing nutrient solution but this method was not very favourable, because we could not get a straight tap root Macht soaked his seeds in tap-water for a night and the following day the swollen seeds were planted with the hilum downwards in finely ground sphagnum moss On the third day the seeds were found to give roots of convenient length and these were hung in plain test-tubes Macht's method did not suit our experiments, so we have tried different kinds of apparatus for germinating the seeds, but the one that suited us best was the germinating apparatus devised by Schonjahn (Plate I fig 2) Seeds of uniform size and weight were taken and were soaked for about two hours in tap-water These were picked up and placed with the hilum turned downwards over the sieve in the Schonjahn's germinating apparatus The sieve has a hundred holes The seeds were then covered over with sand and moistened with water A cover of circular felt was put over it to keep up the necessary warmth A thermometer was fixed over the cover which gives the reading of the temperature at which the seeds are growing Three-fourths of the container was filled up with water The photograph indicates the different parts of the apparatus (Plate I, fig 2) After 24 hours we could see the roots beautifully displayed, growing straight downwards as shown in Plate I, fig 2 The felt covering was then lifted up and all the seedlings were thrown into a tray containing water Seedlings having almost the same length of radicle or root were selected

The other apparatus that we devised allows a straight growth of the radicle The following is its description (Plate I, fig 3) It consists of a fairly wide glass test-tube of 12 mm diameter and of 6 inches height A narrow glass tube of about 5 mm diameter and of 5½ inches length is taken At one end of it a small rubber ring is fitted This rubber ring serves two purposes One in keeping the tube straight when it is put in the test-tube and the other in allowing a good stage for the seed to rest after the narrow tubes are put into each of the test-tubes, the latter have been filled with the physiologic nutrient solution up to the rubber ring The nutrient solution employed is Sach's solution which contains

Pot nitrate	1 gramme	To this a weak solution of ferric chloride is added
Sodi chloride	5 grams	
Calc sulphate	5 grams	
Calc phosphate	5 grams	
Dist water	1,000 c c	

This nutrient solution when diluted with an equal quantity of distilled water seemed best to promote growth of our seedlings In determining this we had occasion to compare the rates of growth of *Vigna catiang* in pure distilled water and in Sach s solution both in the incubator at 37°C and outside at 29°C to 32°C under identical conditions This work occupied a considerable amount of our time

Different sets of 6 tubes each were taken using Sach's solution for one set as controls and in the other sets different sera of pernicious anæmia, secondary anæmia and normal serum in varying dilutions 1 in 50 to 1 in 100 for each, and in some 1 in 200 Thus altogether four sets of tubes are always put up (Plate II, figs 4 and 5)

(1) Sach's solution alone
(2) Sach's solution plus P A serum 1 in 50 dilutions
(3) Sach's solution plus P A serum 1 in 100 dilutions
(4) Sach's solution plus S A serum 1 in 50 dilutions
(5) Sach's solution plus S A serum 1 in 100 dilutions

By previous determination by clinical and other laboratory tests conducted at the General Hospital, Madras, a distinction was made between secondary anæmia and pernicious anæmia Clinical conditions combined with achlorhydria a high colour index, a low halometer reading (Eve's) and an indirect positive van den Bergh were the criteria employed in our diagnosis of a case of pernicious anæmia The secondary anæmia serum was invariably taken from persons that had ankylostome infection to avoid any doubt of the nature of the anæmia Normal serum was taken from healthy individuals and also at times from patients in the hospital that came for some ailment other than anæmia and weakness The blood is drawn from a vein with a sterile syringe and put in a long sterile capsule It is allowed to clot and the serum is gently separated from the clot with a long pipette and put in a long sterile capsule This was sealed with wax and allowed to remain in the frigidaire Some difficulty is always experienced in separating the sera in case of pernicious anæmia Sometimes a certain amount of lysis is seen in the tubes containing the blood of pernicious anæmia The serum used for our experimental purposes was the one taken the previous day We had occasion to compare the results of fresh sera with sera that have been kept in the frigidaire for over a week Our results in every one of our experiments are identical and therefore we are encouraged to collect sera and preserve them for our further experimental study We always kept a certain amount of serum in stock and found it to be not at all deteriorated or to give results different from those when it is used afresh We carefully selected seedlings with tap roots of almost equal lengths with the idea that the stimulus of germination in all of them was given almost at the same time Our experience is that though selected seedlings of the same weight are taken, when allowed to germinate under identical conditions, some did not develop the same length of root as the others during the same period This we believe to be due to

the delay of the stimulus received by the seed to germinate After selecting the seedlings, each of them is transferred to the rubber ring stage of the tubes either with the aid of a forceps or fingers The seed rests upon the ring and the radicle dips into the solution With a grease pencil a mark is made on all the test-tubes, the mark indicating the correct position of the tip of the root The whole apparatus is left in a large wire cage at the room temperature In our earlier experiments we were put to the necessity of using the wire cage because we were troubled by rats completely eating away all the germinating seeds At the end of 24 hours the elongation of the radicle of the respective seedlings was measured and marked The growth of the seedlings in the several tubes was taken from day to day for three consecutive days, and arranged in tabular form After the third day we found a few secondary rootlets growing and obscuring the main root and in some the tap root almost reached the bottom of the test-tube, especially in the control tubes We are convinced that by the third day we could pronounce our results Our readings are taken from the bottom of the rubber ring to the tip of the main root Daily growths of all the seedlings are noted in the controls and in the different sera The following are some of our readings (*vide* Protocols I, II, III, IV, V, VI and VII)

In order to eliminate every source of error and more particularly the personal factor in the measurements of the root, samples of different sera were given to the second author without the clinical and laboratory diagnosis of the case from which they were obtained It is very gratifying to note the data obtained from the experiments are in accordance with our clinical and laboratory findings

In all we dealt with about 32 cases Pernicious anæmia 9, secondary anæmia 10, and normal 13 cases For pernicious anæmia serum with different strengths, 18 sets of tubes were made use of, for secondary anæmia 20 sets, and for the normal 26 sets We found almost in every one of our experiments the growth of the root in the sera of pernicious anæmia distinctly retarded, secondary anæmia allowed a fairly long growth of the tap root and in the normal sera the growth was normal as compared with the growths containing nutritive solution alone In all the experiments the nutritive salts are exactly the same, the seedlings are kept under exactly the same conditions and so any difference in growth is therefore to be attributed to the unknown factors in the sera of pernicious anæmia cases With the data of the measurements of the roots before us, for convenience of expression the ratio of growth in any unknown medium as compared with the growth of the control seedlings in normal Sach's solution is expressed in percentages and is spoken of as the index of coefficient of growth The average coefficient of growth in normal blood serum taken from 13 cases is found to range from 69 to 86 per cent On the other hand the indices of growth obtained with samples of pernicious anæmia are different It was found to range from 25 to 45 per cent giving an average coefficient of 35 per cent Such a retardation in growth indicates fairly conclusively that the blood of patients suffering from pernicious anæmia contains some

toxic substance or something lacking in it that is not conducive to the growth of the radicle Our experiments as those of Macht clearly point out the experimental evidence of the presence of the toxin or a deficiency in the disease, though the nature of the toxin or deficiency is so far unknown The difference between the normal serum and that of pernicious anæmia is so striking that the second author could diagnose the latter condition, when unknown samples of blood are given to him for experimental purposes We could further say that the serum of the secondary anæmia could also be diagnosed from normal and pernicious anæmia cases Therefore we agree with Macht that the 'phytopharmacologic examination not only has been found useful in demonstrating the toxic ætiology of primary anæmia but is also of practical aid in the differential diagnosis of that condition' We have also found that the serum of pernicious anæmia retains its toxicity for a long time when kept in the frigidaire

An attempt made with the kind assistance of Dr M J S Pillai, Radiologist, Government *X*-Ray Institute, Madras, to detoxicate this poison in the sera by ultra-violet radiations with plain glass test-tubes exposed to the radiations at different time intervals on consecutive days has not given us satisfactory results The cause of such we discovered to be due to the small amount of the area of the surface exposed to the radiations and to not using quartz containers, glass being impermeable to ultra-violet radiations We hope to repeat these experiments using quartz containers for irradiation purposes

Our investigations must still be regarded as in the experimental stage but we are convinced that more information will be forthcoming if this line of inquiry is undertaken

Our thanks are due to Mr C J Jayadev, M A , Demonstrator in Biology, for his assistance We also thank Colonel G E Malcolmson, I M S , Professor of Medicine, and First Physician, General Hospital, Madras, and Colonel J M Skinner, I M S , Superintendent, General Hospital, Madras, for their keen interest in the experiments and encouragements and suggestions they had given Our thanks are also due to Dr M J S Pillai for his help in securing the photographs

PROTOCOL I

24 hours	48 hours	72 hours	96 hours	Average	Percentage of coefficient of growth
		Controls			
0 9	1 4	1 7	4 2		
1 3	1 4	1 4	4 1	2 1	100
1 3	1 4	1 2	5 0		

PROTOCOL I—*concld*

24 hours	48 hours	72 hours	96 hours	Average	Percentage of coefficient of growth
		Normal Serum 1–100 *dil*			
1 1	1 2	1 5	3 8		
1 0	1 3	1 6	3 9	1 9	90
1 0	1 0	1 4	3 6		
		Secondary Anæmia Serum 1-100 *dil*			
0 8	0 8	2 2	1 5		
0 6	0 6	1 8	1 0	1 2	57
0 8	0 7	1 9	1 2		
		Pernicious Anæmia Serum 1–100 *dil*			
0 5	0 3	0 2	*nil*		
0 4	0 3	0 1	0 1	0 2	19
0 4	0 2	*nil*	*nil*		

Tables showing the growth at different hours in different sera and in controls (measurements in cms)

PROTOCOL II

48 hours	72 hours	96 hours	Average	Percentage of coefficient of growth
		Controls 50 *per cent Sach's*		
2 8	3 1	3 2	3 03	
2 3	4 4	[4 5	3 71	100
2 9	4 2	[3 7	3 6	
3 1	3 7	4 3	3 7	
			3 6	
		Normal Serum 1–100 *dil*		
1 4	2 5	3 8	2 6	
1 6	2 7	4 0	2 8	76
1 2	2 4	3 7	2 6	
1 7	2 7	4 2	2 9	
			2 7	

PROTOCOL II—*concld*

48 hours	72 hours	96 hours	Average	Percentage of coefficient of growth
		Normal Serum 1-200 *dil*		
17	28	39	28	78
16	27	40	28	
			28	
		Secondary Anæmia Serum 1-100 *dil*		
14	17	22	18	53
15	18	25	19	
12	17	21	19	
12	16	22	19	
			182	
		Secondary Anæmia Serum 1-200 *dil*		
14	18	25	19	56
16	19	27	21	
			20	
		Pernicious Anæmia Serum 1-100 *dil*		
15	08	05	09	22
14	06	02	07	
16	07	04	09	
13	06	02	07	
			08	
		Pernicious Anæmia Serum 1-200 *dil*		
16	11	06	08	26
17	13	06	09	
			086	

PROTOCOL III

24 hours	48 hours	72 hours	Average	Percentage of coefficient of growth
		Controls		
2 5	3 2	4 0	3 3	100
2 4	3 0	3 9		
2 6	3 5	4 2		
2 6	3 6	4 4		
2 4	3 2	4 1		
2 6	3 4	4 2		
		Secondary Anæmia Serum 1–200 *dil*		
0 8	1 4	2 2	1 7	52
1 2	1 6	2 4		
1 2	1 6	2 6		
1 1	1 7	2 8		
		Secondary Anæmia Serum 1-100 *dil*		
0 6	1 7	3 3	1 9	57
0 5	1 4	3 0		
0 8	1 9	3 6		
		Pernicious Anæmia Serum 1–200 *dil*		
1 8	0 5	0 3	1 2	36
2 1	0 7	0 4		
3 1	0 8	0 3		
		Pernicious Anæmia Serum 1–100 *dil*		
1 2	0 3	0 1	0 7	21
1 3	0 2	*nil*		
1 4	0 6	0 2		

PROTOCOL IV

24 hours	48 hours	72 hours	96 hours	Average	Percentage of coefficient of growth
		Controls			
29	37	26	27	30	100
29	32	33	29		
30	31	30	27		
29	35	28	28		
		Secondary Anæmia Serum 1-50 *dil*			
25	14	11	21	17	56
23	12	12	22		
20	14	18	18		
		Secondary Anæmia Serum 1-100 *dil*			
28	14	15	20	19	63
30	20	17	19		
29	20	14	18		
		Pernicious Anæmia Serum 1-50 *dil*			
13	14	05	*nil*	0 61	20
12	11	03	*nil*		

PROTOCOL V

24 hours	48 hours	72 hours	Average	Percentage of coefficient of growth
		Controls		
35	30	21	29	100
28	35	26		
36	34	17		
35	32	17		
32	36	19		
28	36	18		

PROTOCOL V—*concld*

24 hours	48 hours	72 hours	Average	Percentage of coefficient of growth
	Secondary Anæmia Serum 1–50 *dil*			
20	18	02		
30	17	06	15	51
31	16	07		
	Secondary Anæmia Serum 1–100 *dil*			
38	14	06		
34	12	04	17	55
34	10	03		
	Unknown Serum 1–50 *dil*			
33	12	01		
25	16	03	14	48
16	19	04		
	Unknown Serum 1–100 *dil*			
35	21	02		
29	15	02	17	58
29	20	03		
	Pernicious Anæmia Serum 1–50 *dil*			
13	05	00		
11	06	00	06	20
12	07	02		
	Pernicious Anæmia Serum 1–100 *dil*			
19	05	01		
18	06	00	08	27
16	07	02		

PROTOCOL VI

24 hours	48 hours	72 hours	96 hours	Average	Percentage of coefficient of growth
		Controls			
45	46	21	17		
32	36	27	22		
33	33	26	11	31	100
35	39	24	12		
31	40	22	19		
39	31	27	24		
		Tap water			
29	33	22	10		
32	40	30	10		
32	45	25	14	26	84
34	37	25	14		
32	42	16	10		
30	40	12	15		
		No 1 *Serum* 1-50 *Secondary anæmia*			
26	23	03	02		
27	20	03	01	12	39
22	13	02	01		
		No 1 *Serum* 1-100 *Secondary anæmia*			
36	17	07	03		
32	12	03	04	16	51
39	18	16	11		
		No 2 *Serum* 1-50 *Secondary anæmia*			
32	14	02	01		
37	24	16	04	15	48
32	17	11	01		
		No 2 *Serum* 1-100 *Secondary anæmia*			
36	28	05	02		
35	32	02	01	18	57
38	26	14	04		

PROTOCOL VII

24 hours	48 hours	72 hours	96 hours	Average	Percentage of coefficient of growth
		Controls			
3 2	4 0	1 2	1 4	2 7	100
3 3	3 2	2 1	1 6		
3 4	3 2	2 3	1 4		
3 0	3 9	2 2	1 4		
3 2	4 0	2 2	1 5		
3 6	3 9	2 4	1 2		
		Pernicious Anæmia 1–50 *dil*			
1 0	1 2	0 6	0 4	0 8	29
1 1	1 2	0 7	0 2		
1 0	1 1	0 6	0 3		
		Pernicious Anæmia 1–100 *dil*			
1 6	1 1	0 9	0 5	1 0	36
1 8	1 3	0 7	0 4		
1 8	1 4	0 9	0 3		

Fig 1—1 Seeds of *Vigna catiang*
2 Seedlings of the same at different hours of growth

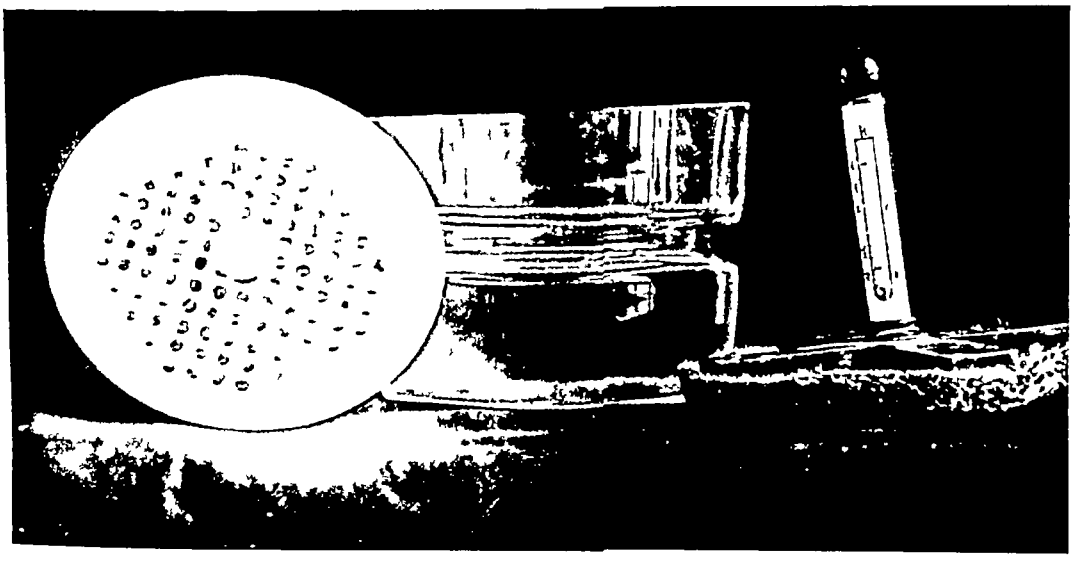

Fig 2—Schonjahn's apparatus in 3 parts
1 Porcelain sieve plate 2 Glass bowl 3 Felt cover with thermometer

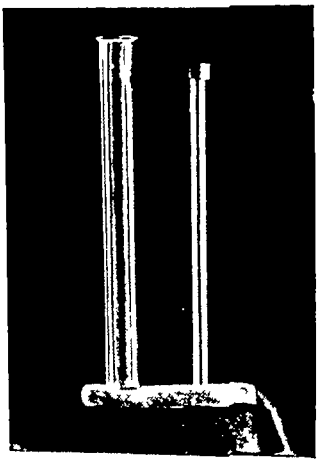

Fig 3—Apparatus devised by the author

NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

Part XXX.

DIAGNOSTIC TABLE FOR THE FEMALES OF THE SPECIES RECORDED FROM INDIA

BY

LIEUT.-COLONEL J. A. SINTON, M.D., D.Sc., I.M.S.

(*From the Malaria Survey of India, Kasauli*)

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MORE than 20 years ago Annandale (1910) drew up a diagnostic table for the seven Indian species of *Phlebotomus* then known to science. This table was based upon such characters as colour differences and variations in wing venation, features now known to be unreliable in many cases. Sinton and Barraud (1928) constructed tables for the differentiation of the females of the five common 'erect-haired' species which appeared likely to be associated in India with the transmission of disease to man, namely *P. sergenti*, *P. papatasii*, *P. major*, *P. argentipes* and *P. chinensis*. These tables were based on such reliable characters as the morphology of the pharynx and the spermathecæ. There are however now on record 27 species and 6 varieties of *Phlebotomus* from India but no table has been published to include all these. Since the synonymy of the Asiatic species was cleared up (Sinton, 1928) and more complete examinations have been made of the different species, it is now possible to make a comprehensive table to include all the females of this genus recorded from India.

The Indian species of *Phlebotomus* may be placed in 3 main divisions —

A. *The Erect-haired Division*

The members of this division have always some erect hairs on the dorsal aspect of segments II to VI of the abdomen *. The heavily chitinized parts of the bases

* Erect hairs occur on the dorsal aspect of the 1st abdominal segment of all the recorded Indian species.

of the spermathecæ of the females of this division are segmented in their entire lengths* (cf Plate III, figs 4, 10, 12, etc)

The species in this division may be arranged into 2 groups —

Group 1 The erect abdominal hairs are numerous, usually occurring in tufts at the distal ends of the segments, dorsal recumbent hairs very scanty or absent on abdominal segments II to VI, buccal armature and pigmented area absent or very rudimentary, the spermathecal chitinizations and male hypopygium usually having specific characters and the pharyngeal armature showing a useful diagnostic morphology This group includes —

1 *P papatasii* Scopoli, 1786
2 *P argentipes* Annandale and Brunetti, 1908
,, var *marginatus* Annandale, 1910
3 *P major* Annandale, 1910
,, var *griseus* Annandale, 1911
4 *P chinensis* Newstead, 1916
5 *P sergenti* Parrot, 1917
,, var *alexandri* Sinton, 1928
6 *P newsteadi* Sinton, 1926
7 *P colabœnsis* Young and Chalam, 1927
8 *P maynei* Sinton, 1930 †
9 *P eleanoræ* Sinton, 1931 †

Group 2 In this group are included the species with scanty erect hairs on the dorsal aspect of abdominal segments II to VI, such hairs are often confined to segments II and III more especially in the male, dorsal recumbent hairs usually numerous, the buccal armature and pigmented area usually well developed and with specific morphology In this group are the following species —

10 *P hospiti* Sinton, 1924
11 *P christophersi* Sinton, 1927
12 *P clydei* Sinton, 1928

B *The Recumbent-haired Division*

In this division the dorsal abdominal hairs on segments II to VI are all recumbent, the body of the spermathecal chitinizations usually with a smooth outline (Plates IV and V, figs 40 and 47) and any traces of segmentation, if present, are confined to the distal end (Plate IV, fig 29) The buccal armature and pigmented area are usually well developed and have a specific morphology This division contains two groups ·

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* The term ' spermathecæ ' in the diagnostic table refers to these chitinizations (*vide* Appendix I)

† The females of *P maynei* and *P eleanoræ* have not yet been recorded

Group 3 In these the morphology of the male genitalia closely resembles that of *P minutus* Rond This group consists of —

13 *P minutus* Rondani 1843
, var *antennatus* Newstead, 1912
14 *P babu* Annandale, 1910
,, var *nigri* Annandale, 1911
15 *P africanus* Newstead, 1912
16 *P montanus* Sinton 1924
17 *P shortti* Adler and Theodor, 1927
18 *P barraudi* Sinton, 1929
19 *P baghdadis*, Adler and Theodor, 1929
20 *P barlyi* Sinton, 1931
,, var *campester* Sinton, 1931

Group 4 The morphology of the male genitalia in this group is distinctly different from that of *P minutus* Rond The group includes —

21 *P zeylanicus* Annandale 1910
22 *P himalayensis* Annandale, 1910
23 *P malabaricus* Annandale, 1910
24 *P sylvestris* Sinton, 1924
25 *P puri* Sinton, 1931
26 *P arboris* Sinton, 1931

C *Intermediate Group*

Only one species, *P squamipleuris*, is contained in this group This species may show a few erect hairs on the dorsal aspect of some of the abdominal segments from the IInd to VIth, but more usually all the hairs are recumbent in this position The spermathecal chitinizations are not crenulated but show a series of transverse rows of short spines (Plate IV, fig 25) This species differs from all the other described species in that the thoracic pleuræ carry tufts of broad scales like those seen in mosquitoes, in the unilateral geniculate spines on the antennæ of the female, in the absence of these spines on segment IV and in the presence of Newstead's sensory spines on both the 2nd and 3rd palpal segments

27 *P squamipleuris* Newstead, 1912

The study of sandflies in India has been confined to a few workers, chiefly on account of the difficulties met with in making accurate determinations of species It is hoped that the table published here will help to solve this difficulty and will lead to a more extensive study of this important genus To facilitate this work among students unfamiliar with these insects, an explanation of the terms used in the table is given in Appendix I and some notes on the mounting and study of

specimens are given in Appendix II A rough guide to the recorded distribution of the different species in India is given in Appendix III *

TABLE FOR THE IDENTIFICATION OF THE FEMALES OF THE INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS* †‡

1	Species with erect hairs on the dorsal aspect of some of the abdominal segments from II to IV §‖	2
	Species with no erect hairs on the dorsal aspect of any of the abdominal segments from II to VI §¶	13
2	Species with numerous erect hairs on the dorsal aspect of the abdominal segments from II to VI, buccal armature and pigmented area poorly developed or absent **	3
	Species with scanty erect hairs on the dorsal aspect of some of the abdominal segments from II to VI	10
3	Spermathecæ short and sausage shaped, each with not more than 8 segments (Plate III, fig 4)	4
	Spermathecæ longer, with characteristic morphology and each with more than 8 segments (Plate III, figs 8, 10, 12, 13, 16 and 18)	5
4	Third antennal segment distinctly longer than half length of proboscis (Plate III, fig 2), wings voluminous, lateral pharyngeal teeth not very markedly oblique (Plate III, fig 5), spermathecæ with 3–5 segments (Plate III, fig 4)	**P. sergenti.**
	Third antennal segment distinctly shorter than half length of proboscis (Plate III, fig 3), wings small, lateral pharyngeal teeth with distinct oblique inclination (Plate III, fig 6), spermatheca with 6–8 segments	**P. sergenti** var **alexandri.**
5	Body of spermatheca moniliform in its entire length (Plate III, fig 8), pharyngeal armature well developed (Plate III, fig 7)	**P. newsteadi.**

* The author will be glad to give any assistance in the identification of specimens sent to him

† Workers should make themselves thoroughly familiar with the explanation of terms used in the Table by a careful study of Appendix I

‡ To facilitate the identification of specimens which have been poorly mounted, in most instances several diagnostic characters have been given As it is difficult to give a written description of many of the characters, the workers should consult the figures on all occasions

§ Read carefully the note on erect and recumbent hairs given in Appendix I

‖ The bodies of the spermathecæ in all these species is markedly segmented, except in *P squami pleuris* This last species has occasionally a few erect dorsal hairs, hence it has been included in two places in the Table, *vide* Nos 10 and 19

¶ The bodies of the spermathecæ in these species is mainly smooth and oval or sausage shaped (Plates IV and V, figs 40 and 47) If any traces of segmentation are present they are confined to a few faint striations near the apical end (Plate IV, fig 29) The undescribed female of *P arboris* probably belongs to this group

** The undescribed females of *P maynei* and *P eleanoræ* belong to this group

	Body of spermatheca not moniliform (Plate III, figs 10, 12, 13, 16 and 18)	6
6	Spermatheca with large 'head' and no 'neck' (Plate III, fig 10), pharyngeal armature a network of fine wavy transverse lines (Plate III fig 9)	**P. papatasii.**
	Spermatheca with small 'head' set on 'neck' (Plate III, figs 12, 13, 16 and 18)	7
7	Spermatheca with long 'neck' (Plate III, fig 12), pharyngeal armature consisting of short transverse lines made up of fine points (Plate III fig 11)	**P. major.***
	Spermatheca with short 'neck' (Plate III, figs 13, 16 and 18), pharyngeal armature appearing as long continuous transverse curved lines at base with obvious teeth about the middle line anteriorly (Plate III, figs 14 and 15)	8
8	Spermatheca fusiform with striations forming triangular areas at margins (Plate III, fig 13), pharynx large and markedly flask shaped with armature consisting of large teeth about middle line and smaller teeth laterally (Plate III, fig 14)	**P. chinensis.**
	Spermatheca carrot shaped with transverse striations (Plate III, figs 16 and 18), pharynx not markedly flask shaped, with armature showing small teeth about middle line and few or none laterally (Plate III, fig 15)	9
9	Spermatheca very markedly carrot shaped with large apical segment (Plate III, fig 16), short spermathecal ducts, each only about twice length of body of organ, sternal tubercle narrow (Plate III, fig 17), geniculate spines comparatively short, Newstead's spines scattered over middle third of 3rd palpal segment (Plate III, fig 16A)	**P. argentipes.†**
	Spermatheca not so markedly carrot shaped with small apical segment (Plate III, fig 18), long spermathecal ducts each about 4 times the length of the body of the organ, sternal tubercle broad (Plate III, fig 19), geniculate spines long, Newstead's spines grouped near middle of 3rd palpal segment (Plate III, fig 17A)	**P. colabaensis**
10	Spermatheca turnip shaped and unsegmented (Plate IV, fig 25), well developed buccal and pharyngeal armatures (Plate IV, figs 24 and 26), groups of wide scales on thoracic pleurae, antennal formula 1 over IV–XV,	

* Annandale (1911) described a variety of *P major* which is 'distinguished from the typical form by the general greyish or brownish (instead of golden) colour', 'the two varieties are easily distinguished by the naked eye' This dark form he named *P major* var *griseus* There appear to be no morphological differences between this and the type form

† Annandale (1911) described a colour variety of this species, *P argentipes* var *marginatus*, which is 'distinguished from the typical form by the fact that the dorsum of the thorax is brown instead of black and the sides of the thorax darker than in the typical form' No morphological differences have been determined to separate this variety from the type form

	Newstead's spines present on both 2nd and 3rd palpal segments (*vide* No 10)	**P. squamipleuris.**
	Spermatheca elongated, sausage shaped with about 8–10 segments (Plate III, fig 21)	11
11	Buccal armature, pigmented area and pharyngeal armature poorly developed (Plate III, fig 20)	**P. christophersi.**
	Buccal armature and pigmented area well developed (Plates III and IV, figs 23 and 28)	12
12	Buccal armature with about 10–15 teeth and pigmented area with pointed tail (Plate III, fig 23), poorly developed pharyngeal armature (Plate III, fig 22)	**P. clydei.**
	Buccal armature with about 50–60 teeth and large pigmented area with truncated tail (Plate IV, fig 28), better developed pharyngeal armature (Plate IV, fig 27)	**P. hospitii.**
13	Pigmented area poorly developed or absent (Plate IV, figs 30 and 35) *pharyngeal armature poorly developed* as transverse ridges with small teeth, or sometimes a few longer spines	14
	Pigmented area well developed	16
14	Buccal armature consisting of a single regular row of teeth and with narrow elongated pigmented area (Plate IV, fig 30), spermatheca may show a few faint apical striations (Plate IV, fig 29)	**P. purii.**
	Buccal armature consisting of 2 or 3 rows of small scattered teeth (Plate IV, fig 35), spermatheca resembling that of *P montanus* (Plate V, fig 47)	15
15	Pigmented area absent (Plate IV, fig 35)	**P. bailyi.**
	Pigmented area small, angular, solid looking	**P. bailyi** var **campester.**
16	Buccal armature with marked notch on posterior border of buccal plate (Plates IV, figs 32, 33 and 34), pharyngeal armature with numerous long teeth (Plate IV, fig 31)	17
	Buccal armature with no notch on plate (Plate IV, figs 36 and 37 and Plate V, figs 43, 45, 48, etc)	18
17	Buccal armature with about 30 teeth (Plate IV, fig 32)	**P. babu.***
	Buccal armature with about 16–18 teeth (Plate IV, fig 33)	**P. baghdadis.†**
	Buccal armature with about 10–14 teeth (Plate IV, fig 34)	**P. shortti.†**
18	With pharynx markedly dilated posteriorly (Plate IV, figs 26, 41 and 42)	19
	With pharynx not so markedly dilated (Plate V, figs 44, 46, 49, 51, 53, 54 and 56)	21

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* Annandale (1911) described a variety of this species, *P babu* var *niger*, 'which is darker than the typical form and as a rule larger' This variety was taken at Pusa in Bihar and an examination of similar specimens from this place revealed no structural differences from the type species

† These two species are closely related to *P babu*, but on account of their morphology and distribution they have been retained as species and not placed as varieties

19	Pigmented area long and oval or hemispherical and buccal armature with convexity backwards also marked lateral buccal protuberances (Plate IV, fig 24), pharyngeal armature with long thin teeth (Plate IV, fig 26), spermatheca turnip shaped (Plate IV, fig 25) (*vide* also No 10)	**P squamipleuris.**
	Pigmented area short and oval, buccal armature with concavity backwards no marked lateral buccal protuberances (Plate IV, figs 36 and 37) short stout pharyngeal spines (Plate IV, figs 41 and 42), spermatheca pipe shaped (Plate IV fig 10)	20
20	With no deep notch on posterior border of pharynx (Plate IV, fig 41), IIIrd antennal segment long (more than 90μ) (Plate IV fig 39)	**P. minutus.**
	With deep notch on posterior border of pharynx (Plate IV, fig 42), IIIrd antennal segment short (less than 90μ) (Plate IV, fig 38)	**P. minutus** var **antennatus**
21	Buccal armature with 12–20 teeth (Plate V, figs 43 and 48)	22
	Buccal armature with more than 20 teeth (Plate V, figs 45, 50, 52, 55 and 57)	23
22	Buccal teeth widely separated, pigmented area broad with short tail (Plate V, fig 43) large wide pharynx markedly constricted posteriorly, showing poorly developed teeth (Plate V, fig 44)	**P. zeylanicus.**
	Buccal teeth in contiguous row, pigmented area narrower with long tail (Plate V, fig 48), small narrow pharynx not markedly constricted posteriorly, showing well developed teeth (Plate V, fig 49)	**P. malabaricus** (?)*
23	With several rows of small lateral teeth in buccal armature, pigmented area with posterior point and broad tail (Plate V, fig 45), pharynx narrow with many teeth (Plate V, fig 46)	**P. montanus.**
	With only one row of buccal teeth (Plate V, figs 50, 52, 55 and 57)	24
24	With numerous long pharyngeal teeth (Plate V, figs 51 and 53)	25
	Without numerous long pharyngeal teeth (Plate V, figs 54 and 56)	26
25	Pigmented area concave posteriorly (Plate V, fig 50) and narrow pharynx with comparatively few teeth (Plate V, fig 51)	**P. africanus.**
	Pigmented area convex posteriorly with small projection (Plate V, fig 52), broader pharynx with many long teeth (Plate V, fig 53)	**P. barraudi.**

* These are the females described by Sinton (1927) as probably *P malabaricus* from Assam This still requires confirmation by the examination of females from the same area where the type males were collected, i e, 'Travancore, S India, below the Western slopes of the Western Ghats'

26	With about 80–100 buccal teeth arranged in a line slightly convex backwards and with triradiate pigmented area (Plate V, fig 55), numerous stout, short, pharyngeal teeth (Plate V, fig 54)	**P. himalayensis.**
	With about 25 buccal teeth arranged in a line distinctly concave backwards and with mop like pigmented area (Plate V, fig 57), ridged pharyngeal armature (Plate V, fig 56)	**P. sylvestris.**

APPENDIX I

Explanation of the Terms used in the Table

A *Erect and Recumbent Hairs*

The hairs are erect on the dorsum of the 1st abdominal segment of all the known Indian species of *Phlebotomus*, while those on the other segments may be all erect, all recumbent or mixed erect and recumbent The character of the hairs on the dorsal aspect of the abdomen from IInd to VIth segments forms a very useful starting point in the identification of the females of the Asiatic species of *Phlebotomus* In unrubbed specimens* this character is easily distinguished, but in specimens in which the dorsal abdominal hairs have been displaced it may be a matter of difficulty This difficulty may, however, be overcome by an examination of mounted specimens under the microscope to determine the nature of the scars left by the detached hairs The scars left by erect hairs are much larger and more refractile than those left by recumbent hairs (Plate III, fig 1) The scars left by the former hairs are also more circular or oval than those of recumbent hairs, which have a more elongated outline (Plate III, fig 1) One can become familiar with the appearances of these scars by comparing those on the dorsum of the abdomen of an erect-haired species (*P papatasii*) with those on a recumbent-haired specimen (*P babu*) or perhaps better by examining the female of a species with both types of hair on the dorsum (*P hospitii*, *P christophersi* and *P clydei*)

B *Sternal Tubercles*

These are two small sickle-shaped tubercles arising from the front of the thorax between the front coxæ (Plate III, figs 17 and 19)

C *Antennæ*

The length of the IIIrd antennal segment has been used in differentiating *P sergenti* from its variety *alexandri* (Plate III, figs 2 and 3) and *P minutus* from its variety *antennatus* (Plate IV, figs 38 and 39)

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* This character is better preserved in specimens stored dry than in those stored in alcohol

The *geniculate spines* are curved spines arising from the sides of the antennal segments (Plate III, fig 2 G) These spines are usually present on the antennal segments from the IIIrd to XVth The 'antennal formula' is expressed by means of a kind of fraction which has as numerator the number of geniculate spines (1 or 2) situated on the different segments and for denominator the numbers in order of the corresponding segments The antennal formula of most of the females of the *Phlebotomus* from India is 2 over III–XV but *P squamipleuris* differs from all the others in that it has a formula of 1 over IV–XV, i e, a single geniculate spines on each segment from the IVth to XVth

D *The Palp*

The 'palpal formula' is made by writing in order of increasing length the number of the palpal segments If two segments are of equal length they are grouped in a parenthesis and are placed in anatomical order and in order of their common size as compared with the other segments

Newstead's spines are 'a compound group of minute and curiously modified spines found on the 3rd palpal segment' They have each usually a long curved pedicle with a spathuliform end (Plate III, figs 16A and 17A) In *P squamipleuris* these spines are found on both the 2nd and 3rd segments of the palp The number, position and distribution of these spines may be useful in diagnosis

E *The Buccal Cavity* *

The points of interest in the buccal cavity are —

(*a*) The *buccal armature* which may consist of (*i*) a single curved row of contiguous teeth (Plates III and IV, figs 23 and 28) or (*ii*) a single row of separate teeth (Plates III and IV, figs 20 and 30) or (*iii*) a single row of contiguous teeth with a double or treble row laterally (Plate V, fig 45) or (*iv*) one or more rows of small scattered teeth (Plate IV, fig 35) The row of teeth is usually arranged with the concavity backwards (Plate IV, figs 32 and 36) but sometimes with the convexity in this direction (Plate IV, fig 24) These teeth lie on the floor of the posterior part of the buccal cavity The buccal armature is very poorly developed or absent in the species with numerous erect hairs on the dorsal aspect of the abdomen, but forms a very useful diagnostic feature in other species

(*b*) The *pigmented area* is an area of dark chitin on the dorsal wall of the cavity overlapping and extending anterior to the buccal armature (Plate III, fig 23 P) Its size and shape are important in diagnosis It is absent in all the species with numerous erect hairs on the abdomen

* The mid pharynx of Patton and Hindle (1928)

(*c*) The *buccal plate* is seen near the roots of the teeth of the armature It has usually a smooth posterior outline with a backward concavity, but in some species it may show a marked notch in the middle line, which is very characteristic of the females of *P babu*, *P baghdadis* and *P shortti* (Plate IV, figs 32 N, 33 N and 34)

(*d*) The *lateral buccal protuberances* (Plate IV, fig 24 L) are seen as marked chitinous masses projecting inwards from the lateral walls of the buccal cavity anterior to the armature They are well marked in *P squamipleuris*

F *The Pharynx* *

This organ forms the backward continuation of the buccal cavity and varies in size, shape and the degree of expansion of the posterior end (Plate IV, figs 27, 31 and 42 and Plate V, fig 44) It is composed of three chitinous plates and in transverse section it is triangular, the dorsal plate lying horizontally The posterior parts of all three plates carry an armature composed of ridges or spines, but in speaking of the ' pharyngeal armature ' that of the dorsal plate is understood

The *pharyngeal armature* may consist of (*a*) a series of transverse lines composed of fine points (Plate III, fig 11) or (*b*) a series of transverse ridges which may carry a few small blunt teeth at their free edges (Plate V, figs 44 and 56) or (*c*) teeth of varying size and shape projecting backwards (Plate III, figs 5, 14 and 15, Plate IV, figs 26, 27, 31 and 41 and Plate V, fig 47)

G *The Spermathecæ*

These organs lie in the posterior part of the abdomen They each consist of a single thin-walled, sub-spherical sack and are relatively very large, at their junction with the duct they are strongly chitinized The structure of this chitinization of the organ shows a characteristic morphology in many of the species of the ' erect-haired group ' (Plate III, figs 8, 10, 12, etc) In speaking of the spermathecæ in the diagnostic table this chitinization is referred to In all the Indian species which have erect hairs on the abdomen, except *P squamipleuris* (Plate IV, fig 25), the chitinous part of the organ shows segmentation in its entire length In the recumbent-haired division this portion is thin-walled and usually smooth (Plates IV and V, figs 40 and 47) but occasionally a few faint traces of striation may be seen near its apex (Plate IV, fig 29)

The spermathecal ducts may open separately into the vulva, as in *P chinensis* and *P papatasii*, while in other species they may unite to form a common duct before reaching the surface, as in *P major*, *P argentipes* and *P colabaensis* The relative lengths of these ducts may be helpful in diagnosis

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* Jobling (1928) states that morphologically this portion of the alimentary tract is really the œsophageal pump not the pharynx

APPENDIX II

Methods of Collection, Storage and Mounting of Specimens

The following notes are given in the hope that they may aid workers in the study of 'sandflies'*

(a) *Collection*

These insects are of very small size (about 1·5 to 3 mm in length) and of an inconspicuous greyish, yellowish or brownish colour Many species do not appear to bite man but to feed on other mammals, birds or reptiles If one of the local species has a predilection for the human host, its presence is usually brought to notice by its vicious biting propensities, but in areas where such species are rare 'sandflies' may be reported as scanty or absent Their small size makes them easily overlooked The position of the wings, which are in most species carried erect and divergent at an angle of about 45 degrees to the body of the insect, as well as their curious hopping flight, makes their appearance characteristic After a few specimens have been carefully observed in the live state, there is usually no tendency to confuse them with any of the other small insects which are found inside buildings

These insects are usually inactive during the day, but in darkened rooms they may bite at any time In well-lighted habitations during the day-time they usually take refuge behind pictures, clothing, wardrobes, etc, or retire into cracks in the walls A favourite habitat is in bathrooms, where the combination of moisture with comparative darkness seems especially congenial to them Badly lighted and poorly ventilated cowsheds and stables are other favourite haunts Some of the wild species are collected in hollow trees or cracks in rocks Some species, such as *P squamipleuris*, are strongly attracted by artificial light, while others will collect on the walls just outside the area of intensive light from a lamp

If living specimens are required, a wide-mouthed test-tube about 1 to 1½ inches in diameter and 7 or 8 inches long has been found the most suitable thing in which to catch these insects When the insect has been covered by the tube, if a piece of stiff paper or cardboard is slipped carefully between the tube and the wall, the insect is secured alive and dealt with as desired The aspirator described by Buxton (1928) is also a very useful instrument

If living insects are not required the specimens caught by any of the above methods may be killed with tobacco smoke, but for routine work the tube described by Christophers, Sinton and Covell (1931) seems the handiest These tubes are prepared as follows —'Take a large test-tube with a well fitting cork, and place a

* In an earlier publication (Sinton, 1925) these methods were described As this publication is not always available and certain improvements in technique has been evolved since that time, the methods are again summarized here

number of rubber rings or small pieces of rubber tubing in the bottom A few c cms of chloroform are poured down the side of the tube and are absorbed by the rubber, any excess that may remain after a few minutes is poured off A small plug of cotton-wool wrapped in gauze is pushed down upon the rubber rings, and above this a thin perforated disc, cut from the cork which is used to close the tube The latter is a useful addition, as it makes it easy to transfer the dead insects to another receptacle by simply inverting the test-tube A tube thus charged will remain effective for some weeks Insects should not be left in the tube longer than is necessary to kill them, but when dead should be transferred to an ordinary specimen tube with a numbered cork ' or to the storage tubes described below

(b) Storage

The best method of storage, in my experience, has been to use a small piece of glass tubing about 1½ inches long One end is plugged with cotton-wool and the dead insects are poured into the open end This is then closed by pushing another piece of wool into it with a match just sufficiently far to prevent the insects shaking about without crushing them The cotton-wool at each end of the tube is touched with a little carbolic acid to protect the specimens

A piece of paper with a pencilled note of the locality, date and any other essential details is pasted on the outside of the tube The tubes can be stored in cigarette tins, and, as an additional precaution against moulds and predatory insects, a piece of cotton-wool soaked in creosote or pure carbolic acid can be placed in the tin along with the tubes

Some workers recommend the preservation of specimens in 70 per cent alcohol This method has not been found satisfactory and seems of use only when specimens are required for section, etc Insects preserved in spirit are liable to lose their colour They also become very brittle so that the appendages and hairs are often detached in the spirit It has been my experience that specimens preserved for any length of time in this manner are very difficult to clear with caustic potash (*vide* infra)

(c) Mounting and Preparation for Identification

The specimens for identification may be either (1) fresh, (2) dry, or (3) preserved in spirit or other fixative The method of mounting may be either temporary or permanent

A note should be made, before mounting the specimens, of the colour of the insects, of the presence of scales on any portions of the body where they are not usually found, such as the abdomen, pleuræ, etc , whether the hairs on the dorsum of the abdomen are erect or recumbent, the character and arrangement of the antennal hairs and similar points of diagnostic importance A binocular dissecting microscope is very useful for this purpose

(1) Examination of fresh specimens

(a) Temporary methods In the case of 'sandflies' of unknown identity required for dissection to detect the presence of parasites, the method adopted is that recommended by Sinton and Barraud (1928) —

'The fly is shaken up in a small quantity of saline solution in a test-tube to render it thoroughly wet and to remove as many of the hairs as possible This method also kills the insect and avoids the use of chloroform which is undesirable for this purpose when dissections are to be carried out The insect is then placed in a drop of normal saline solution (0 6 per cent) on a slide With dissecting needles the head is carefully separated from the thorax by placing one needle across the base of the head and the other across the front of the thorax exerting traction on the head in a forward direction If carefully done, the anterior portion of the alimentary tract will be drawn out, still attached to the head and unbroken mid-gut' The terminal segments of the abdomen are now dissected by nicking the chitin with a needle on either side of the abdomen near the genitalia and gently drawing out the hind-gut and the spermathecæ The hind-gut is cut through close to the anus and the terminal segments of the abdomen with the attached spermathecæ are placed in a drop of saline of lacto-phenol * 'The remainder of the alimentary tract is drawn out through the thorax by exerting traction on the head in a forward direction Should it be necessary to dissect the head to determine the presence of parasites in the anterior part of the alimentary tract, the head capsule and appendages are removed so as to leave the pharynx, buccal cavity and biting mouth parts only, attached to the rest of the intestinal canal When a cover-glass is applied, the specific morphology of the buccal cavity, and pharyngeal armature is easily determined in the saline solution'

In the case of flies not required for dissection, the method of identification is as follows —

'The fresh insect is placed in a drop of lacto-phenol and the head and terminal segments of the abdomen are separated from the body The head is arranged ventral side uppermost, a cover-glass applied and slight pressure exerted If examined under a $\frac{1}{6}$th inch objective, the arrangement of the lines and teeth at the base of the pharynx (and the morphology of the buccal cavity) can usually be clearly seen, especially if the preparation is allowed to clear for a short time The terminal segments of the abdomen are treated in the same way to demonstrate the form of the spermathecæ, which in case of difficulty can be found by following the spermathecal ducts'

* Amann's lacto phenol solution consists of carbolic acid (chemically pure crystals) one part, glycerine two parts, lactic acid one part and distilled water one part Liquid carbolic acid may be used but is not so good

In case it is desired to make permanent preparations of specimens which have been temporarily mounted, the specimens are washed into a watch-glass and treated with caustic potash, etc , as described below Specimens mounted in lacto-phenol solution may be preserved by ringing with du Noyer's mixture,* but this is not so good as the method of mounting in Canada balsam

(*n*) Permanent methods

(*a*) The dead specimen is placed in a watch-glass and wetted with a single drop of 70 per cent alcohol †

(*b*) The watch-glass is immediately filled with a 10 per cent aqueous solution of caustic potash, in which the insect is allowed to remain overnight (12 hours or longer) ‡

(*c*) Next morning the insect is washed by placing it in 3 or 4 changes of water, each of which is allowed to act for at least 15 to 30 minutes to get rid of all the alkali §

(*d*) After thorough washing, the water is pipetted off and replaced by a 20 per cent aqueous solution of Ziehl-Neelsen's carbol fuchsin mixture,‖ which is allowed to act for about 12 hours or overnight ¶

(*e*) Pipette off half the stain and add an equal amount of absolute alcohol Allow this to act for about half an hour

(*f*) Pipette off the solution and replace with 70 per cent alcohol

(*g*) Dehydrate in absolute alcohol **

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* du Noyer's mixture consists of anhydrous lanoline 20 parts and colophane 80 parts

† This makes it sink in the caustic solution, otherwise the numerous hairs make it liable to float on the surface and maceration does not occur so well

‡ This softens the dry insect, makes the appendages, which have become contracted and distorted in drying, swell up to their normal size and shape, and at the same time detaches the majority of hairs which otherwise obscure many of the important details in stained specimens It also dissolves out all the non-chitinous matter and exposes the chitinous structures which are so important in diagnosis This step may be hastened by boiling the insect in the caustic solution for some minutes and allowing it to stand in the hot fluid Better preparations are usually obtained by the slower method It is also an advantage, when the insect has become softened by the caustic solution, to prick the chitin of the abdomen and thorax in a couple of places to facilitate the entry of the fluid into the body

§ It is preferable not to transfer the specimen from one receptacle to another but to keep it in the same vessel until ready to transfer it to balsam The different fluids are pipetted off and replaced in the various steps of the technique

‖ Ziehl Neelsen's stain consists of basic fuchsin one part, absolute alcohol 10 parts and aqueous solution of carbolic acid (5 per cent) 100 parts

¶ This step can be hastened by gently heating the specimen in the pure stain till the steam rises and allowing it to soak in the hot stain for several minutes This process is repeated two or three times, but seldom gives as good preparations as the slower method

** In damp climates it will be found that amyl alcohol forms a much more satisfactory dehydrant than absolute ethyl alcohol

(*h*) Replace the alcohol with oil of turpentine or xylol* and allow to clear

(*i*) The insect is transferred to a drop of *thin* Canada balsam on a clean slide

(*j*) Two methods may be used for the further arrangement of the specimen on the slide —

Method A The head is cut off and transferred to a small drop of thin balsam about an inch from one end of the slide The head is turned ventral side uppermost, the appendages displayed and the preparation covered with a small cover-glass The spermathecæ are drawn out as described in the preparation of temporary specimens and the detached terminal abdominal segments and spermathecæ are removed to a drop of thin balsam about an inch from the other end of the slide, where they are arranged and covered with a small cover-glass The remainder of the insect is displayed and covered By this method it is usually possible to make good preparations, but better ones can be made by method B which is more difficult to carry out

Method B In this method the buccal cavity, pharynx and spermathecæ are dissected out For this purpose a dissecting microscope is needed and very fine dissecting needles

The insect in thin balsam is turned on its side, the thorax is steadied with one needle and the clypeus is transfixed from side to side with the other needle If gentle traction is used with the clypeal needle it is possible to draw out the buccal cavity and pharynx with the attached hypopharynx This manœuvre requires practice and, if skilfully done, should do no damage to any part of the insect except the clypeus The extracted parts are placed in a small drop of balsam about an inch from one end of the slide

Turn the insect on its back, being careful not to damage any of the appendages in doing so This is liable to occur if the balsam has become thick and sticky Make a small nick on either side of the abdominal chitin just proximal to the hypopygium and, steadying the insect with the other needle, draw the genitalia away with the attached spermathecæ These parts are transferred to a small drop of thin balsam near the other end of the slide The remainder of the insect is turned on its side and, after arranging the wings and other appendages, is covered with a cover-glass

Under the dissecting microscope the buccal cavity and pharynx are arranged with their dorsal aspects uppermost and allowed to become fixed in this position by the drying of the balsam When they are fixed in the proper position a small cover-glass† is wetted in xylol and dropped over them This glass is gently pressed

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* Turpentine does not seem to make the specimens so brittle as does xylol The specimen is left in the turpentine until clear, or, if overstained, until it is seen under the microscope to be sufficiently decolorized

† These are made by cutting a larger glass into 4 small squares with a writing diamond

into position under the dissecting microscope so that the diagnostic characters are displayed In a similar manner the spermathecæ are mounted *

2 Examination of dry specimens

Dry specimens are wetted with alcohol and macerated with caustic solution as for fresh specimens After washing they can be made into temporary preparations by the lacto-phenol method or stained and mounted in Canada balsam as described above

3 Examination of specimens preserved in spirit

These may be treated like dry specimens but do not clear so well in the caustic solution as do specimens which have not been fixed in spirit

APPENDIX III

Geographical Distribution of Indian Species of *Phlebotomus*

The geographical distribution of the different Indian sandflies has not yet been worked out with any great degree of accuracy, but the following notes may serve as a guide to the records at present available

(*a*) *P papatasii* is scattered all over the plains of India, more especially in those areas where the climate is hot and dry It has been found as far east as Calcutta and as far south as Madras City but is most common in the north-western parts of the country This species is the carrier of sandfly fever and is believed to transmit Oriental sore in the Mediterranean basin

(*b*) *P argentipes* This species is mainly confined to the area east and south of a line joining Bombay and Simla, but a focus has been found in Kathiawar It has also been reported at Rangoon It prefers a warm moist climate and has been found at altitudes as high as 4,000 feet (Sanawar, Simla Hills) It is suspected to be the carrier of kala-azar

(*c*) *P major* seems essentially a hill species in India, at heights of 5,000 to 7,000 feet in areas where there is a marked rainfall in summer It appears to occur all along the Himalayan foot-hills

(*d*) *P chinensis* has a distribution similar to that of *P major*

(*e*) *P sergenti* seems confined to the plains on an area north and west of a line joining Bombay and Simla It is found under conditions like those in which *P papatasii* occurs Its distribution resembles that of Oriental sore *P sergenti* var *alexandri* has only been recorded from the western frontier of India

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* It is an advantage not to press the glass too tightly on the specimen, because, if a sufficient layer of balsam lies between the glass and the slide, old specimens which have become displaced can be manœuvred into position again by gently heating the slide and, when the balsam is soft, re arranging the specimen by a gentle movement of the cover-glass

(*f*) *P newsteadi* is a hill species like *P major*

(*g*) *P colabaensis* has only been recorded from Bombay City and from Bissum Kuttack in the Vizagapatam Agency of Madras

(*h*) *P maynei* has been found in Saharanpore, U P, and *P cleanorae* at Karnal, Punjab

(*i*) *P hospita* has only been recorded in the western foot-hills of the Himalayas

(*j*) *P christophersi* has been found chiefly in the Punjab

(*k*) *P clydei* is recorded widely from the plains of India

(*l*) *P minutus* occurs chiefly in the north-western plains of India, while its variety *antennatus* has a more general distribution over the plains of India

(*m*) *P africanus* has been found in Sind

(*n*) *P babu* seems to have a very wide distribution over the plains and foot-hills of the country

(*o*) *P baghdadis* has been found in the western and north-western areas

(*p*) *P shortti* has been recorded from the north-eastern parts of India and from Burmah

(*q*) *P barraudi* has only been found in Assam and Burmah

(*r*) *P barlyi* has a very wide distribution from sea-level up to 6,000 feet

(*s*) *P montanus* occurs in the western foot-hills of the Himalayas at about 6,000 feet

(*t*) *P zeylanicus* has been found in many widely separated areas in India It appears to be a wild species chiefly associated with jungle and with moist warm conditions

(*u*) *P malabaricus* The type males of this species were collected in Travancore but no further specimens have been recorded from that area Females believed to belong to this species have been found in Assam and at Saharanpore, U P

(*v*) *P sylvestris* has been recorded from Sukna in the Darjeeling District, where it seems associated with jungle in the foot-hills Specimens have also been received from Sylhet and Rangoon

(*w*) *P himalayensis* has only been found at Kurseong (5,000 feet) in the eastern Himalayas

(*x*) *P puri* was found in tree-holes in the forest at the base of the Himalayas in the Darjeeling District and *P arboris* was collected under similar conditions

(*y*) *P squamipleuris* has a very wide distribution all over India both on the plains and in the foot-hills up to 6,000 feet It is a garden species

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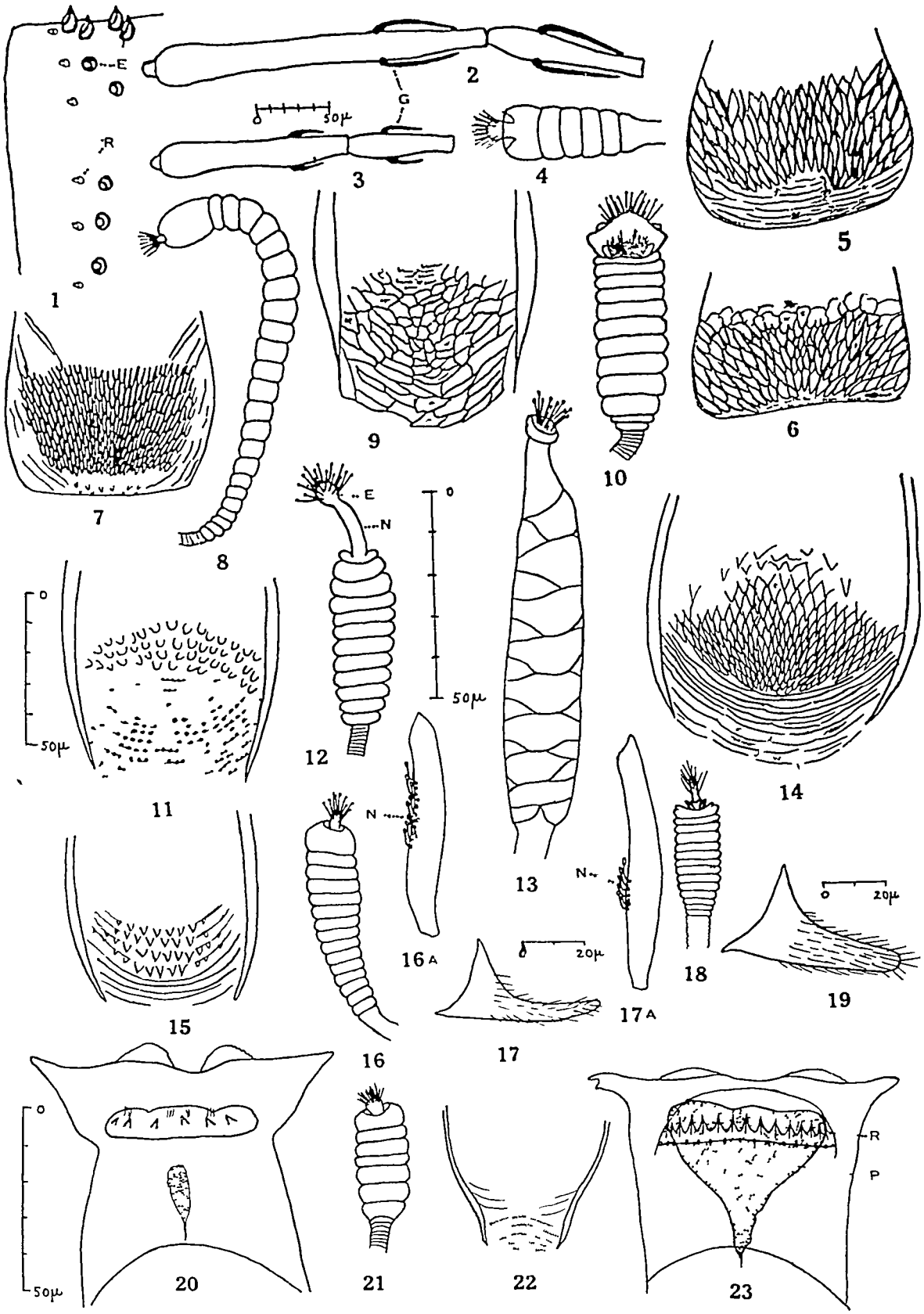
EXPLANATION OF PLATE III

All the spermathecæ are drawn to the scale shown opposite Fig 12 The scale for the buccal armatures is shown beside Fig 20 and that for the pharyngeal armatures is beside Fig 11

Fig 1 Dorsum of abdominal segment of *P hospitii*, showing the scars left by erect hairs (E) and by recumbent hairs (R)
,, 2 Third and fourth antennal segments of *P sergenti*
,, 3 Third and fourth antennal segments of *P sergenti* var *alexandri* G—geniculate spines
,, 4 Spermatheca of *P sergenti*
,, 5 Pharyngeal armature of *P sergenti*
,, 6 Pharyngeal armature of *P sergenti* var *alexandri*
, 7 Pharyngeal armature of *P newsteadi*
,, 8 Spermatheca of *P newsteadi*
,, 9 Pharyngeal armature of *P papatasii*
,, 10 Spermatheca of *P papatasii*
,, 11 Pharyngeal armature of *P major*
,, 12 Spermatheca of *P major* E—head, N—neck
,, 13 Spermatheca of *P chinensis*
,, 14 Pharyngeal armature of *P chinensis*
,, 15 Pharyngeal armature of *P argentipes*
,, 16 Spermatheca of *P argentipes*
,, 16A. Third palpal segment of *P argentipes* N—Newstead's spines
,, 17 Sternal tubercle of *P argentipes*
,, 17A Third palpal segment of *P colabaensis*
, 18 Spermatheca of *P colabaensis*
,, 19 Sternal tubercle of *P colabaensis*
,, 20 Buccal cavity of *P christophersi*
,, 21 Spermatheca of *P christophersi*
,, 22 Pharyngeal armature of *P clydei*
,, 23 Buccal cavity of *P clydei* R—buccal armature, P—pigmented area.



J A SINTON del

Explanation of Plate IV

The spermathecæ are drawn to the scale shown opposite Fig 29 The scale for the buccal armatures is that shown beside Fig 36 and that for the pharyngeal armatures is beside Fig 41

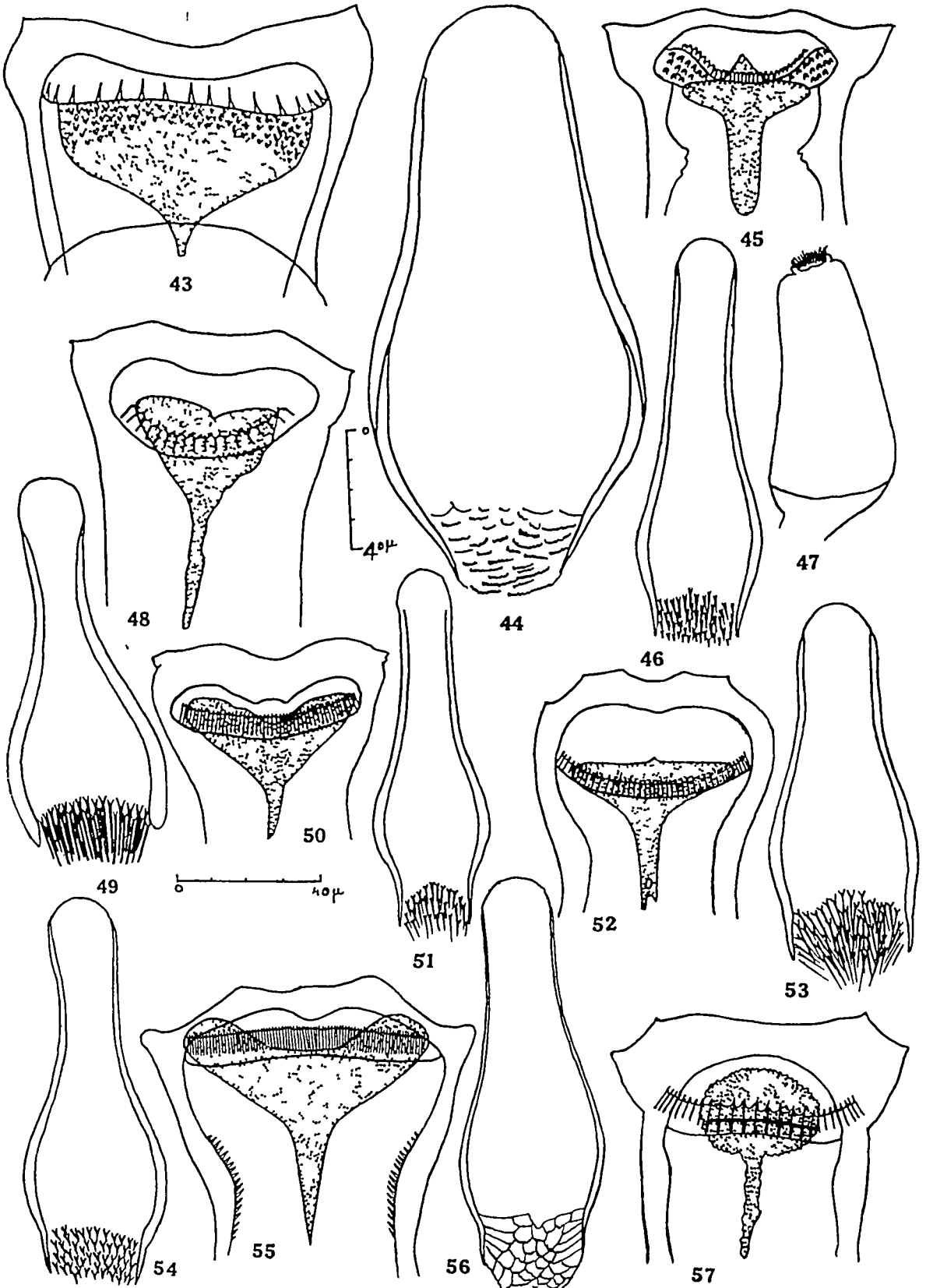
Fig 24 Buccal cavity of *P squamipleuris*
„ 25 Spermatheca of *P squamipleuris*
„ 26 Pharynx of *P squamipleuris*
„ 27 Pharynx of *P hospitii*
„ 28 Buccal cavity of *P hospitii*
„ 29 Spermatheca of *P purii*
„ 30 Buccal cavity of *P purii*
„ 31 Pharynx of *P babu*
„ 32 Buccal cavity of *P babu* N—notch in buccal plate
„ 33 Buccal cavity of *P baghdadis*
„ 34 Buccal cavity of *P shortti*
„ 35 Buccal cavity of *P bailyi*
„ 36 Buccal cavity of *P minutus*
„ 37 Buccal cavity of *P minutus* var *antennatus*
„ 38 Third and fourth antennal segments of *P minutus* var *antennatus*
„ 39 Third and fourth antennal segments of *P minutus*
„ 40 Spermatheca of *P minutus*
„ 41 Pharynx of *P minutus*
42 Pharynx of *P minutus* var *antennatus*

Explanation of Plate V

All the buccal cavities are drawn to the scale shown below Fig 50 and the pharyngeal armatures to that beside Fig 44

Fig 43 Buccal cavity of *P zeylanicus*
„ 44 Pharynx of *P zeylanicus*
„ 45 Buccal cavity of *P montanus*
„ 46 Pharynx of *P montanus*
„ 47 Spermatheca of *P montanus*
„ 48 Buccal cavity of *P malabaricus* (?)
„ 49 Pharynx of *P malabaricus* (?)
„ 50 Buccal cavity of *P africanus*
„ 51 Pharynx of *P africanus*
„ 52 Buccal cavity of *P barraudi*
„ 53 Pharynx of *P barraudi*
„ 54 Pharynx of *P himalayensis*
„ 55 Buccal cavity of *P. himalayensis*
„ 56 Pharynx of *P sylvestris*
„ 57. Buccal cavity of *P sylvestris*

PLATE V



J A SINTON *del*

LEAD IN URINE

BY

LIEUT.-COLONEL T C BOYD, I.M.S.,

AND

H D GANGULY, M.Sc.

(From the Department of Chemistry, Medical College, Calcutta)

[Received for publication, February 24, 1932]

UNTIL comparatively recent years the question of lead poisoning was of little importance in India, times are, however, changing and latterly we have had several samples of urine sent to the laboratory for analysis for this element. Our results may prove of interest to others engaged on this question and we hope in addition that they may be of considerable use to members of the Medical Profession who send in samples.

ANALYTICAL METHODS FOR THE DETECTION OF LEAD

Broadly speaking these may be classified into —

(1) Chemical methods

(2) Micro-chemical methods

(3) Spectrographic methods

A perusal of the current literature will impress the enquirer with the many methods that can be applied to this problem under the heads 1 and 2. In this paper, however, we propose to restrict ourselves purely to the micro-chemical tests and particularly to the triple nitrite test of Behrens and Kley, in which the presence of lead is proved by the formation of the crystalline compound $K_2Cu\ Pb(NO_2)6$ readily recognized under the microscope. Lawrence T. Fairhall of the Harvard Medical School has given an excellent account of his method in applying this test to clinical material (*Journal of Biological Chemistry*, 57, 1923) and we have followed closely his technique in the actual application of the test itself.

GENERAL PROCEDURE ADOPTED

(1) *Collection of the urine* This was collected direct where possible into Pyrex flasks which before despatch to the Wards were prepared by treating them with 100 c c of lead-free hydrochloric acid specific gravity 1 124 and heating on the waterbath for three hours, then subsequent treatment with dilute nitric acid for one hour and finally washing with double distilled water and draining Officers sending in specimens to the Laboratory appear to neglect the importance of clean lead-free containers and possible contaminations from urinals, etc The urine should also be freshly collected as we find that when fresh it ashes more easily We need not refer again to the importance of the preparation of the glassware used in the test, nor to the necessity of being certain that all reagents are lead-free

(2) *Ashing of the urine* One litre of the urine is evaporated to dryness in a porcelain basin on the waterbath and the residue completely ashed in a silica or porcelain crucible the former is preferable The time required to ash the residue obtained from one litre takes about twenty-five hours over the Bunsen flame

(3) *Fairhall's application of the Behrens Kley triple nitrite test* The ash obtained is moistened with distilled water, twenty-five c c and fifteen c c of pure hydrochloric acid specific gravity 1 124 added, this treatment does not cause complete solution of the ash, a little white gelatinous matter is left undissolved A few drops of methyl orange are now added as an indicator and excess of acid neutralized with ammonium hydroxide to a final pH of approximately 3 8 Particular care must be taken to adjust the reaction or the lead will not be completely precipitated The whole is now transferred to a centrifuge tube and freed by spinning from the undissolved precipitate (We have found that examination of the gelatinous precipitate usually reveals the presence of lead where the final result of the test is positive) Two c c of a saturated solution of ammonium sulphate and a drop of a two per cent copper acetate solution are now added and the whole saturated with sulphuretted hydrogen for at least half an hour The precipitated sulphides are separated by the centrifuge and thoroughly washed three times with double distilled water, the final wash water is removed by means of a capillary pipette The tube containing the precipitate is now placed in a beaker of boiling water and two drops of nitric acid specific gravity 1 140 added followed by two drops of water When solution is complete the whole is removed and evaporated to dryness on a microscopic slide, when dry 0 005 c c of a four per cent sodium acetate solution is added and evaporation to dryness again carried out The slide is now allowed to cool at room temperature, when cool the residue is treated with 0 005 c c of acetic acid and a small crystal of potassium nitrite Five minutes are allowed to elapse when the slide is examined under the microscope for the characteristic crystals The examination should not be delayed too long as we find that in this hot damp climate the crystals are liable to disappear One other point that requires attention is that when dissolving the precipitated sulphides with nitric acid, the tube should not

be left in the boiling water longer than one minute as some of the sulphur is oxidized to sulphuric acid which interferes with the test

Sensitivity of the test Experimenting with known dilutions of lead in water, we found this test to be sensitive to 0 00003 mg , the figure given by Behrens and Kley

Results of our investigations

Series I —*European Group*

The following series of European urines were examined from hospital patients These were kindly supplied by Colonel Acton, I M S , and Dr Ferens Coltman

(*a*) Patient E E Occupation, Wireless Operator Disease Fracture of Tibia and Fibula Result Lead present

(*b*) Patient K Occupation, Mining Engineer Disease Ankylostomiasis Result Lead negative

(*c*) Patient K Occupation, Sailor Disease Dysentery Result Lead present

(*d*) Patient V A Occupation, Sailor Disease Malaria Result Lead present

(*e*) Patient C Occupation, Bank Official Disease Amœbiasis Result Lead present

(*f*) Patient J Occupation, Sailor Disease Ulcer of leg Result Lead present

(*g*) Patient C Occupation, Sailor Disease Duodenal ulcer Result Lead present

(*h*) Patient S Occupation, Engineer Disease Urticaria Result Lead negative

(*i*) Patient N Occupation, Engineer Disease Amœbiasis Result Lead present

(*j*) Patient E Occupation, Missionary Disease Dysentery Result Lead present

(*k*) Patient L Occupation———— Disease ———— Result Lead present

(*l*) Patient M Occupation, Office Worker Disease ———— Result Lead present

Series II —*Indian Group*

(*a*) Brahmins Six different individuals of this class were examined using one litre of urine from each Result Lead negative in all

(*b*) Baidyas and Kyasthas Six individuals examined using one litre of urine from each Result Lead negative in all

object of our investigation was to find out if the excretion of lead was common in this country and, if so, was it equally distributed amongst the different classes of the community Our results show that amongst Europeans it is extremely common but that where Indians are concerned the amount of lead excreted in the urine falls outside the limit of sensitivity of the method employed Why there should be this difference we are unable at the moment to explain and can only record our findings

Summary and conclusions

We have recorded the result of examinations made on urine for the determination of lead on various classes of the community and as far as Europeans are concerned this appears to be commonly present while in the Indian groups of the community the excretion of this element appears to lie outside the sensitivity of the method employed

We have quoted the findings of workers employed on this question in other countries

Finally we have drawn attention to the fact that the views of certain investigators appear to indicate very strongly that the detection of lead in urine or even its quantitative estimation is of very doubtful value as far as the diagnosis of lead poisoning is concerned

THE RELATIVE VALUES OF MEINICKE MICRO-FLOCCULATION TEST AND WASSERMANN REACTION BASED ON A COMPARISON OF 1,200 SERA

BY

MAJOR R C WATS, M D, D P H, D T M, I M S,

AND

ASSISTANT SURGEON C M E EYLES, I M D

(*From the District Laboratory, Maymyo, Burma*)

[Received for publication, February 27, 1932]

IT IS NOW UNIVERSALLY ADMITTED that in order to get most reliable information regarding sero-diagnosis of syphilis, at least two different methods should be used, one of which should preferably be the Bordet-Wassermann reaction In India, however, there is another point to be considered, the laboratories carrying out the Wassermann tests are few and far between The cost (usually Rs 32) prevents its universal application for diagnostic purposes in civil practice and even in cases where the patients can afford the fee valuable time is lost in obtaining the result, as the test is carried out once a week only The complexity of the Wassermann reaction stimulated various workers to discover simpler flocculation methods but, in our opinion, there is none simpler, more economical and least time consuming than the Meinicke's micro-flocculation test, which could be adopted alone or in addition to the complement deviation test, as a routine by any ordinarily equipped clinical laboratory in the tropics

The principle of the test—The antigen consists of an extract of ox-heart diluted with alcohol to which balsam of tolu has been added On admixture with a 3 5 per cent solution of sodium chloride which has been made alkaline with sodium carbonate, the antigen forms a densely milky, turbid and opaque mixture The latter, on addition of a positive syphilitic serum gives rise to flocculi which can be seen under the low power of the microscope

TECHNIQUE

The patient's serum —The blood from a finger prick is collected in a capillary tube about 10 cm in length and having an inside diameter of a millimetre or so Two-thirds of the tube is filled and the empty end sealed in a flame particularly avoiding heating the blood The tube is left for a few hours in a cool place in a horizontal position to allow the separation of the sera This process may be expedited by centrifugalizing towards the sealed end The sealed end is broken and the serum after separation is blown or tipped on to a numbered slide The sera are not inactivated but the latter can also be used for the test In the majority of our tests the sera were a portion of those sent to the laboratory for Wassermann test

Antigen —This is best obtained from ADLER-APOTHEKE Otto Steingraber HAGEN, I W, Germany

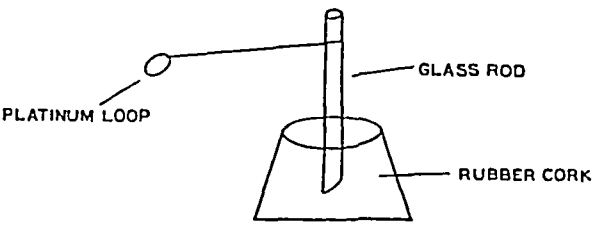
The cork-stoppered bottle should be kept at room temperature in a dark place (*also see* below) As the cost is nominal we have not given the details of the preparation of the antigen

Salt solutions —Two stock solutions are used, namely, (*a*) a 3 5 per cent solution of sodium chloride, (*b*) a 1 per cent solution of sodium carbonate in 3 5 per cent salt solution For the test itself 3 parts (0 1 c c) of stock solution (*b*) are added to 97 parts (3 23 c c) of stock solution (*a*)—this gives a 0 03 per cent soda salt dilution

Of this soda saline mixture 1 c c is taken in a small test-tube, to another tube 0 1 c c of the heart extract is added, both tubes are incubated in a water bath at 45°C (an improvised cigarette tin bath serves the purpose) for 10 minutes They are then mixed by pouring rapidly several times from one to another (to start with adding saline to the heart extract) The resulting extract dilution is then poured in to a *warmed* porcelain crucible covered with a watch-glass and allowed to ripen for two minutes We have found this milky and opaque mixture stable for about twenty minutes and in this period one can, with the help of an assistant, put up about thirty-five tests Here we would like to say a few words about the storage of the heart extract Meinicke recommends it to be stored at room temperature of the laboratory (68°F or so) but in our case, there being no special heating arrangement, the temperature of the storage room fell below 50°F on several occasions In winter we noticed that on mixing the soda saline with the heart extract by pouring rapidly from tube to tube, the resin separated thus rendering the mixture unfit for use, this we ascribe to the difference in the storage conditions On such occasions we gently added the soda saline to the extract by pouring down the sides of the tube and then transferred the mixture without shaking to the warmed porcelain crucible, the rim of the tube touching the rim of the tilted crucible while pouring This mixture worked quite satisfactorily as will be evident by results given below

Mixing —Two platinum eyelets 4 mm and 2 mm in diameter are required for mixing The large one is used for taking up the extract solution and the smaller

for the patient's serum Mixing of the latter with the extract is carried out by passing the smaller eyelet several times rapidly through the lumen of the larger A drop of the mixture from the small eyelet is now placed on a clean cover-slip and a hang-drop preparation made avoiding formation of air bubbles, numbered hollow ground slides previously vaselined together with clean cover-slips are kept ready for this purpose to save time These slides with the serum extract mixture are allowed to stand for one hour at room temperature Memicke advises leaving these slides in a moist atmosphere at 68°F but we have taken no special measures except the steam of the water bath for moistening the atmosphere The temperature in our working room has been below 60°F on several occasions without interfering with the test The platinum eyelets, fixed horizontally on brass holders and mounted on porcelain stands, could be obtained from the address given under antigen or improvised as shown in the diagram



For facility of work two pairs are necessary, the assistant cleaning one pair by first dipping in water, next in alcohol and then flaming them before a fresh serum is put up for the test

Examination —After an hour the slides can be read under the microscope using the $\frac{2}{3}$rd objective ×10 eyepiece, in a few doubtful cases the results can be checked by the $\frac{1}{6}$th objective We found no special difference in the readings taken four hours after putting up the hang-drop preparation

A negative reaction shows an even finely granular field in which the particles show lively Brownian movements

In the strong positives cessation of movement and agglutination of the particles takes place, the large flocculi often forming a network with the interspaces free from granules

In the weak positive reaction the flocculi are smaller and the interspaces may show varying numbers of granules

An intermediate picture with smaller flocculi than the strong positive and fewer granules in the interspaces than the weak positive has been classified by us as *positive*

If the picture shows granulation of various shapes and sizes but no distinct flocculation the reaction is noted as *negative incomplete*

Following the above technique the Meinicke micro-flocculation test was carried out on 1 203 sera and Wassermann method (Medical Research Council method No 1 revised in accordance with the latest published pamphlets on the subject) was adopted for comparative purpose The results are summarized as follows —

Total number of tests —1,203

Agreements	1,079	
Disagreements	124	(10 3 per cent)

The 1,079 agreements have been classified as follows —

Positive agreements 202		Negative agreements 877	
S1a	85	S1a	3
S2a	21	Sp	266
S3a	2	V S	484
Sp	78	Non-Venereal	115
V S	12	Gonorrhœa	9
Others	4		
TOTAL	202	TOTAL	877

Disagreements	124	
Major		59 (4 9 per cent)
In minor degree only		65 (5 4 per cent)

Major disagreements . 59 (4 9 per cent)

	Wassermann Positive Meinicke Negative	Wassermann Negative Meinicke Positive
S1a . ..	6	
S2a ..	4	
Sp	15	18
V. S	5	10
Others	.	1
TOTALS	30	29

In minor degree only 65 (5 4 per cent)

	Wassermann negative incomplete Meinicke negative	Wassermann negative Meinicke negative incomplete	Wassermann positive Meinicke weal positive	Wassermann negative incomplete Meinicke positive
S1a			3	1
S2a			2	
Sp	6	10	8	5
V S	8	9	3	7
Non Venereal	2	1		
TOTALS	16	20	16	13

S1a	Syphilis Primary before treatment
S2a	Syphilis Secondary before treatment
S3a	Syphilis Tertiary before treatment
Sp	Syphilis cases after treatment
Others	Sera sent to exclude Syphilis

The differences both minor and major, after a study of the clinical history sheets, have been classified as follows —

1 *Meinicke lasting longer in cases after treatment*
18 cases accounting for 14 major and 15 minor differences

2 *Wassermann lasting longer in cases after treatment*
15 cases accounting for 19 major and 5 minor differences

3 *Meinicke showing first in untreated cases*
8 cases accounting for 4 major and 6 minor differences

4 *Wassermann showing first in untreated cases*
5 cases accounting for 6 major and 4 minor differences

5 *Primary disagreement followed by agreement*
(*a*) From the study of the cases presumably Meinicke test was faulty
7 cases accounting for 5 major and 2 minor differences
(*b*) From the study of the cases presumably Wassermann test was faulty
5 cases accounting for 3 major and 2 minor differences

6 *Serum septic —Meinicke correct* (from clinical data)
2 cases accounting for 2 major differences

7 *Icteric sera —Wassermann correct* (from clinical data)
2 cases accounting for 2 major differences

8 *Cases not followed due to insufficient tests or data*
6 cases accounting for 4 major and 2 minor differences

Total number of cases	68 accounting for	59 major and	36 minor differences
Balance of cases not accounted for	27 „	„ 0 „	„ 29 „ „
TOTAL	95 „	„ 59 „	„ 65 „ „

It would be seen from the above details that about 90 per cent of the results by Meinicke micro-flocculation test agreed with the Wassermann test Out of the 59 (4 9 per cent) major differences 33 (about 56 per cent) are accounted for by one of the other tests persisting positive after the treatment and ten by one or other of the tests appearing as such earlier Though no special reason can be ascribed eight major disagreements were followed by agreements and in two tests though sera were septic the Meinicke's test was correct Two differences are due to icteric sera in which the Wassermann seemed to be correct (*see* below) and the balance of 4 major differences are unaccounted for It is not possible to conclude which of the tests appears positive earlier before and which persists longer after the treatment The minor differences being of degree only have not been discussed

SUMMARY OF SOME RELEVANT EXPERIMENTS

1 *Rabbits sera*—Sera from 7 rabbits were tested by Wassermann and Meinicke micro-flocculation test There were five negative agreements but in two cases though the former were weak positive, the latter were negative Several workers have drawn attention to the frequent occurrence of Wassermann positive sera in stock rabbits, but in the small number of tests we carried out the Meinicke test seemed to be specific

2 *Septic sera*—Four negative sera were rendered septic by leaving the tubes exposed for a few days, after which both flocculation and Wassermann tests were carried out on the 7th, 14th, 21st and 28th days Meinicke's test was negative throughout but the Wassermann was distinctly positive though the sera were slightly anticomplementary Two similar tests on sera received as a routine in the laboratory have been referred to above

3 *Icteric sera*—Two cases of icteric sera in which the Wassermann was positive and Meinicke's tests negative have been referred to above To investigate the matter further a serum strongly positive to both Wassermann and Meinicke was divided into three parts and marked as '*a*', '*b*' and '*c*' To '*a*' a loopful of sterile ox-bile was added and to '*b*' a loopful of sterile sodium taurocholate solution (5 per cent) The portion '*c*' was left as such to act as a control The sera were examined a week after, the control '*c*' was strongly positive to both tests while '*a*' and '*b*' were positive to Wassermann but negative to Meinicke

Whether it is the surface tension or some other factor at work Meinicke test fails to record positive in the presence of bile salts as also observed in the above two routine sera Meinicke, however, claims that his test is specific for icteric sera as well

SUMMARY

1 The Meinicke's micro-flocculation is a much simpler sero-diagnostic test than the Wassermann

2 The apparatus and ingredients are cheap and easily obtainable

3 The results can be obtained much more rapidly (60 tests requiring a little over an hour)

4 There are 90 per cent agreements with Wassermann results and half of the 10 per cent differences are of minor degree only Ninety per cent of the major differences are accounted for either by one or other of the tests appearing earlier before or persisting longer after the treatment, or followed by agreement with each other in subsequent tests

5 The Meinicke micro-flocculation test gives reliable results with hæmolytic or contaminated (*cf* Wassermann) but not with icteric sera

We wish to acknowledge with many thanks the help received from Assistant-Surgeon I White, I M D , in the early part of this work

Ind Jour Med Res, XX, 1, July, 1932

SOME SOURCES OF VITAMIN C IN INDIA

Part II—(*Continued*)

GERMINATED PULSES, TOMATOES, MANGOES AND BANANAS

BY

MAJOR R C WATS, MD, DPH, DTM, IMS,

AND

ASSISTANT SURGEON C M E EYLES, IMD

(*From the District Laboratory, Maymyo, Burma*)

[Received for publication, March 20, 1932]

THIS investigation is the continuation of the work published in the *Indian Medical Gazette*, **64,** No 2, February, 1929, and the *Indian Journal of Medical Research*, **19,** No 2, October, 1931

Furst (1912) was the first to discover that germination endowed the various seeds with antiscorbutic properties Chick and Hume (1917) confirmed this in the case of peas and lentils, these authors recommended germinated pulses as a valuable means of preventing scurvy in the absence of fresh fruit and vegetables Unlike the latter the pulses require no preservation, can be easily transported and germinated on the spot to serve as a supply of both water soluble vitamins Our investigation was undertaken to make a comparative study of various dals commonly used in India, both as regards the ease with which they can germinate in the limited period of 48 hours (including soakage) and their antiscorbutic values The antiscorbutic va ue of dwarf yellow bananas was described in the second paper referred to above, we have further extended the work by investigating the values of the red and green varieties available in the local market The local tomatoes and mangoes were also assayed

Guinea-pigs, as usual, were used for the experiments, weights and details of post-mortems, etc, are attached as an appendix The basal diet consisting of bran and oats with 30 per cent of crushed gram (*Cicer arietinum*) as described by Wats and White (1931) was used The omission of autoclaved milk makes the diet free

from any traces of vitamin C, which may have survived the heating We have not noticed any appreciable difference in the symptoms of scurvy and the condition of the animals has been quite satisfactory showing no special inanition Owing to the shortage of guinea-pigs we have been obliged to use in some experiments older and heavier animals which is a slight deviation from the normal routine

The details of the articles tested are as follows —

1 PULSES, ETC —These were obtained from the local bazar and were not husked or split The whole peas or pulses were placed between two layers of lint steeped in a large petri dish containing water The soaking was allowed to go on for 24 hours, after which the seeds were spread between two layers of moist lint The germination was allowed to go on for 24 hours at room temperature (average maximum 76°F, average minimum 54°F during the period of the experiment)

The following seeds were tested for their germinating capacities following the above technique —

(*a*) Chana (*Cicer arietinum*) or chick pea
(*b*) Peas (*Pisum sativum*)
(*c*) Mung (*Phaseolus mung*) or green gram
(*d*) Urd (*Phaseolus mung* var *radiatus*) or black gram
(*e*) Lobia (*Vigna catjang*)
(*f*) Soya bean (*Glycine hesepida*)

It was noticed that the percentage of seeds germinating under the above standard conditions of lobia and soya bean was very poor the radicles being hardly perceptible In others 65 per cent seeds germinated of urd, 70 per cent of gram, 75 per cent of mung and 90 per cent of peas The length of the radicle was 4 mm in gram and mung and 6 mm in urd and peas

2 THE GREEN AND RED BANANAS (varieties of *Musa sapientum*) obtained from the local market in Maymyo are similar to those procurable in India The former is about 9 inches in length and comparatively thinner than the latter The differences in pulp are also noticeable being white in the green variety and creamy in the red

3 TOMATO (*Lycopersicum esculentum*) —There are several varieties of this vegetable available locally The one we tested is about the size of a large plum, deep red in colour and uniformly round

4 MANGO (*Mangifera indica*) —The particular fruit we used comes from Mandalay District and is locally called 'Nettiya' In size and flavour it is similar to the 'Langra' variety obtainable in India, similarly it has no fibres in the pulp

The animals under experiment were grouped as follows —

Group 1 —Controls

(*a*) One animal on laboratory diet consisting of crushed gram, bran and oats with vegetable leaves and green grass

(*b*) One animal on the basal diet alone

(*c*) One animal on basal diet with 5 drops of Irradol (vitamin D concentrate prepared by Messrs Parke, Davis and Co)

Group 2 —Pulses, etc

Three animals were put on basal diet supplemented by 3, 4 and 5 grammes each of germinating grams peas, mung, urd and lobia Soya beans were not used as the seeds did not germinate after 24 hours soakage and another 24 hours exposure to a moist atmosphere

Group 3 —Bananas, red and green

Two subgroups of 3 animals each were put on basal diet with 5, 10 and 15 grammes of fruit pulp

Group 4 —Tomato

Three animals were put on basal diet with the addition of 3, 5 and 7 c c of tomato juice

Group 5 —Mango

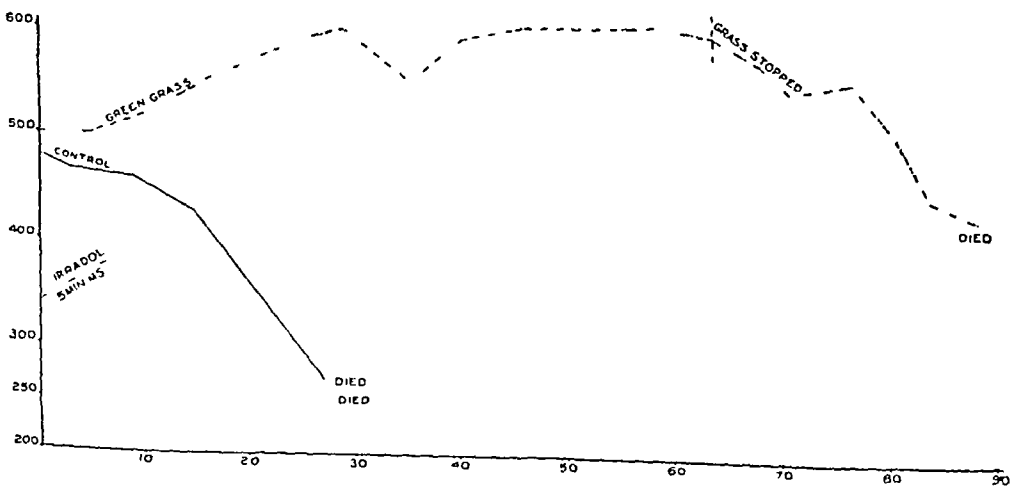
Four animals were put on basal diet with 5, 10, 15 and 20 grammes of the fruit pulp (not juice)

Summary of observations

In the case of *Group* 1 the animal (*a*) on laboratory diet increased steadily in weight until the 63rd day after which the green grass was discontinued, the animal died of scurvy after an interval of 25 days

CHART NO 1

Group 1 —*Controls*



The animal (*b*) on basal diet alone died of scurvy on the 27th day, weighing at death 270 grammes instead of the initial weight of 480 grammes

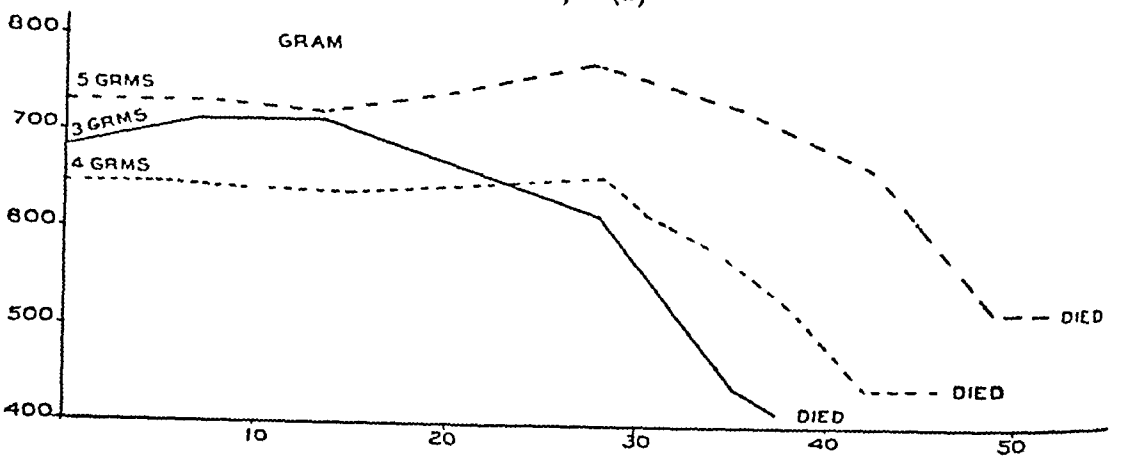
The animal (*c*) on basal diet with Irradol died of scurvy on the 28th day weighing 90 grammes less than the initial weight of 340 grammes

Group 2—Germinated pulses

(*a*) *Gram.*—All the animals (on 3, 4 and 5 grammes) more or less maintained their weights for the first three weeks or so, dying of scurvy respectively on the 39th, 46th and 52nd day

CHART NO 2

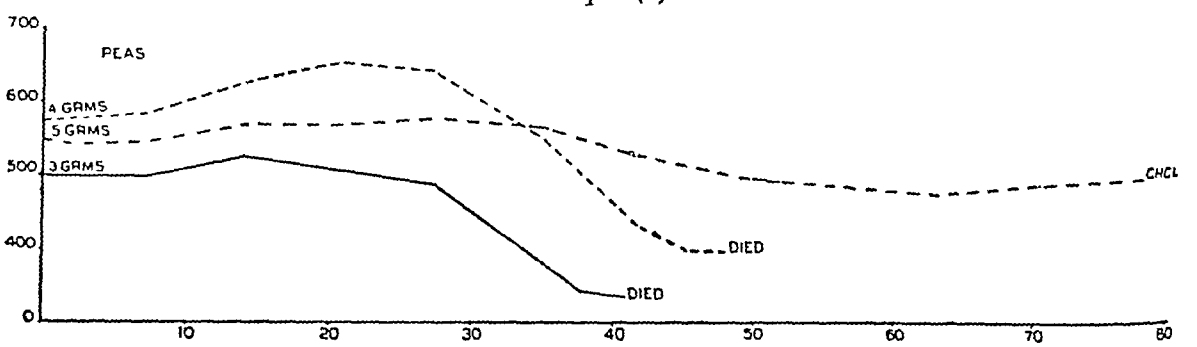
Group 2(*a*)



(*b*) *Peas*—The animals on 3 and 4 grammes, after a preliminary rise, lost weight rapidly dying of scurvy on the 41st and 48th days respectively The animal on 5 grammes more or less maintained its weight losing some at the end of the experiment when it was chloroformed Signs of scurvy were present post-mortem

CHART NO 3

Group 2(*b*)

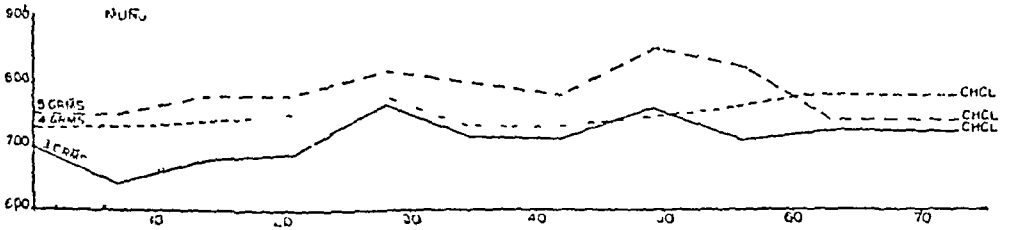


(*c*) *Mung*—The animal on 3 grammes after an initial loss steadily increased in weight until the termination of the experiment on the 73rd day The animals on 4 and 5 grammes put on weight to some extent, the one on 5 grammes showing some loss towards the end. All three animals on being chloroformed showed no signs of scurvy post-mortem

(*d*) *Urd*—The animal on 3 grammes maintained its weight for about four weeks, then showing a decline, on the 36th day it showed a typical 'face-ache' posture, dying on the 39th day

CHART No 4

Group 2(*c*)

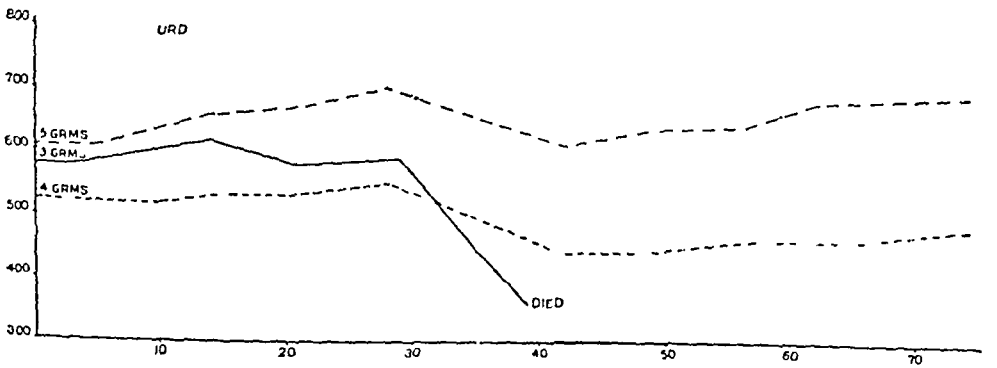


The animal on 4 grammes did not lose any weight for four weeks when it gradually went down On the termination of the experiment (74th day) it had lost 50 grammes of its original weight looking otherwise normal

The animal on 5 grammes increased steadily in weight until the 28th day and at the termination of the experiment showed an increase of 30 grammes on the initial weight The animal was released being in good health

CHART No 5

Group 2(*d*)



(*e*) *Lobia*—The animal on 3 grammes began to lose weight at the beginning of the experiment until its death on the 34th day Signs of scurvy were present post-mortem

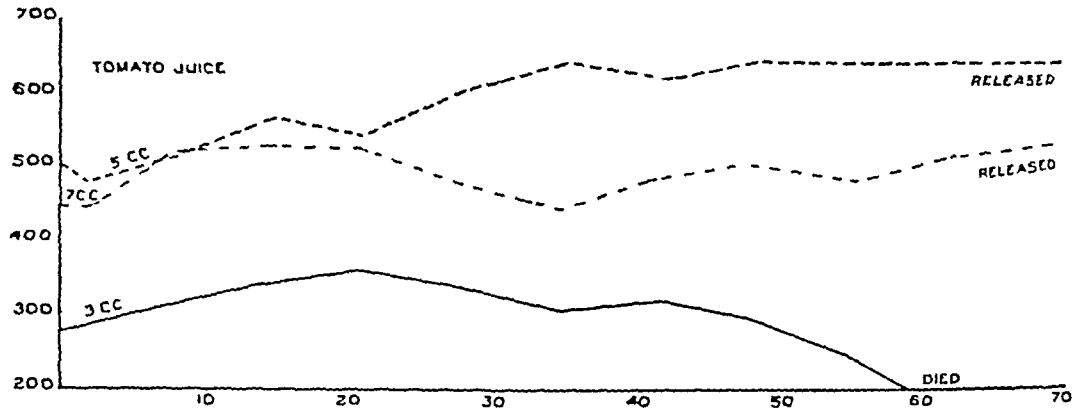
The animal on 4 grammes maintained its weight for a fortnight then began to lose until its death on the 32nd day Signs of scurvy were present post-mortem

The animal on 5 grammes gained slightly in weight, fell ill for a few days on the 40th day losing some weight, after which it gained steadily till the 56th day when it was chloroformed No signs of scurvy were found post-mortem

the 59th day showing signs of scurvy post-mortem The animals on 5 and 7 c c of the juice gained steadily in weight showing slight ups and downs and were released on the 70th day looking quite normal

CHART NO 9

Group 4

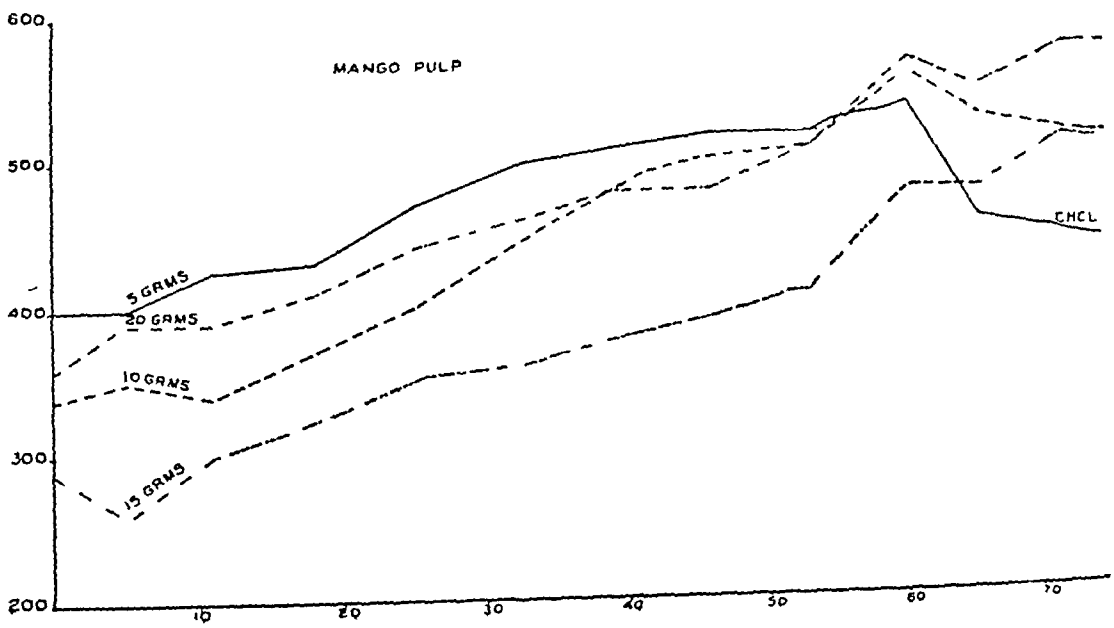


Group 5 —Mango

All the animals did well except the one on 5 grammes which lost a little weight towards the end of the experiment The movements of the hind legs must have been painful as the animal was disinclined to move, it had lost its

CHART NO 10

Group 5



appetite and the condition of its fur was poor On the 74th day the animal was killed showing signs of scurvy The animals on higher amounts were released after the 74th day being in very well-nourished conditions

STATEMENT OF RESULTS

The basal diet —The basal diet with crushed gram worked quite satisfactorily, the animals thrived well without any intestinal disturbances The animal on laboratory diet died after an interval of 25 days after stoppage of green grass, illustrating once again the inability of guinea-pigs to store vitamin C in the body The animal on an excess of vitamin D (in the shape of Ibradol) did not survive much longer than the control animal, neither was there any appreciable differences in special symptoms of scurvy

The pulses, etc —The antiscorbutic value does not seem to depend on the amount of germination or on the length of the radicle Amongst the pulses tested by us, *mung* seems to be the best both as regards its antiscorbutic value as well as the ease with which it germinates A further advantage is that the germinated seeds being tender a salad can be prepared with the addition of sauce, salt and pepper thus eliminating the waste of any vitamin by cooking We have consumed the amount suggested for prevention of scurvy in human beings and found it quite palatable no indigestion or flatulence was noted as an after effect

Bananas, red and green —It was stated in a previous paper (*Indian Journal of Medical Research*, **19**, 2nd October, 1931) that 5 grammes of yellow banana was an adequate amount to prevent scurvy in guinea-pigs , this, however, is not true in the case of red and green bananas Ten grammes at least of red banana pulp were required for the purpose while in the case of green banana pulp even 15 grammes proved insufficient

Tomato —Five c c of the juice were sufficient to act as a prophylactic of scurvy in guinea-pigs The local variety is evidently inferior to those obtainable in England or America

Mango —Ten grammes of the pulp was found adequate to prevent scurvy in guinea-pigs

The evaluation of vitamin C content classified according to the suggestion of Wats and White (1931) has been recorded in the appendix We have to acknowledge with many thanks the help received from Assistant Surgeon I White, I M D , in the investigation of mangoes and bananas

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HURST (1912) *Zeits f Hyg* , **22.**
WATS and WHITE (1931) *Ind Jour Med Res* , **19**, No 2, pp 393—413

APPENDIX No 1

GROUP 1 —CONTROLS

Day of experiment	ANIMAL ON LABORATORY DIET		ANIMAL ON BASAL DIET		ANIMAL ON BASAL DIET WITH 5 DROPS OF IPRADOL	
	Weight	Remarks	Weight	Remarks	Weight	Remarks
1st	500		480		340	
5th	500		460		370	
8th	510		460		390	
15th	550		430		370	
20th	570		360		330	
25th	590		290		290	
27th	590		270	*Died* P-M Showed hæmorrhages round joints, intestines, pleura	260	
28th	590				250	*Died* P M Hæmorrhages round hnd joints, bladder and intestines
30th	590					
35th	550					
40th	590					
45th	600					
50th	600					
60th	600					
63rd	590	Green grass stopped				
70th	540					
76th	550					
80th	500					
83rd	440					
88th	420	*Died* P-M . Hæmorrhages subcutaneous, round joints, bladder, suprarenals Costo chondral joints enlarged				

APPENDIX No 1—*contd*

GROUP 2(*a*)—GRAM (GERMINATED)

Day of experiment	ANIMAL ON BASAL DIET WITH 3 G		BASAL DIET WITH 4 G		BASAL DIET WITH 5 G	
	Weight	Remarks	Weight	Remarks	Weight	Remarks
1st	690		650		730	
7th	710		650		730	
15th	710		640		720	
22nd	660		650		740	
29th	580		640		760	
36th	440	Ailing	550		730	
39th	410	*Died* P M Hæmorrhages round joints, subcutaneous, bladder, intestines, suprarenals Costochondral joints enlarged				
43rd			440	Face ache	670	
46th			440	*Died* P-M Hæmorrhages bladder, intestines, lungs and pleura	580	
49th					520	Ill
52nd					520	*Died* P M Hæmorrhages subcutaneous, round joints, bladder, intestines, and suprarenals Costochondral joints enlarged

APPENDIX No 1—*contd*

GROUP 2(*b*)—PLAS (GERMINATED)

Day of experiment	ANIMAL ON BASAL DIET WITH 3 G		BASAL DIET WITH 1 G		BASAL DIET WITH 5 G	
	Weight	Remarks	Weight	Remarks	Weight	Remarks
1st	500		580		550	
7th	500		590		550	
14th	530		620		570	
21st	510		660		570	
28th	490		650		580	
35th	380	Ill	560		570	
38th	340	Face ache				
41st	340	*Died* P M Teeth loose, hæmorrhages subcutaneous, bladder, testes, kidney, suprarenals Costo chondral joints enlarged				
42nd			430	Face ache	530	
45th			400			
48th			400	*Died* P M Hæmorrhages round joints, subcutaneous and peritoneal Costochondral markedly enlarged and hæmorrhagic		
49th					500	
56th					490	
63rd					490	
70th					490	
78th					500	Chloroformed P M Subcutaneous hæmorrhages, also bladder and suprarenals

APPENDIX No 1—*contd*

GROUP 2(*c*)—MUNG (GERMINATED)

Day of experiment	ANIMAL ON BASAL DIET WITH 3 G		BASAL DIET WITH 4 G		BASAL DIET WITH 5 G	
	Weight	Remarks	Weight	Remarks	Weight	Remarks
1st	700		730		750	
7th	640		730		750	
14th	680		710		780	
21st	690		750		780	
28th	770		780		820	
35th	720		740		810	
42nd	720		740		790	
49th	770		760		860	
56th	720		770		830	
63rd	740		790		760	
70th	740		790		760	
73rd	740	Chloroformed P M No signs of scurvy	790	Chloroformed P M No signs of scurvy	760	Chloroformed P M No signs of scurvy

GROUP 2(*d*)—URD (GERMINATED)

1st	580		530		610	
8th	590		520		620	
15th	620		530		660	
22nd	580		530		670	
29th	590		550		700	
36th	420	Face ache	490		650	
39th	360	*Died* P M Hæmorrhages round joints, bladder Costo chondral joints enlarged, and hæmorrhagic				

APPENDIX No 1—*contd.*

GROUP 2(*d*)—URD (GERMINATED)—*concld*

Day of experiment	ANIMAL ON BASAL DIET WITH 3 G		BASAL DIET WITH 4 G		BASAL DIET WITH 5 G	
	Weight	Remarks	Weight	Remarks	Weight	Remarks
43rd			410		600	
50th			410		620	
57th			460		620	
64th			460		650	
71st			480		640	
74th			480	Released in good health	640	Released in good health

GROUP 2(*e*)—LOBIA (GERMINATED)

1st	760		850		460	
5th	740		840		490	
12th	620		850		520	
19th	640		850		530	
26th	600		770		540	
32nd	460		760	*Died* P M Hæmorrhages, bladder and round joints	500	
34th	440	*Died* P M Hæmorrhages, bladder and suprarenals Costo chondral joints enlarged			480	
41st					430	Ruffled fur
45th					450	
50th					480	
56th			..		490	Chloroformed P M No signs of scurvy

APPENDIX No 1—*contd*

GROUP 3(*a*)—GREEN BANANA PULP

Day of experiment	BASAL DIET WITH 5 G		BASAL DIET WITH 10 G		BASAL DIET WITH 15 G	
	Weight	Remarks	Weight	Remarks	Weight	Remarks
1st	250		300		280	
4th	260		260		290	
10th	220		300		270	
17th	240		330		300	
24th	270		350		330	
31st	280		360		350	
38th	300		340		380	
45th	280		370		355	
52nd	220		400		320	
54th	200	*Died* P M Hæmorrhages round knee joints, testes and intestines				
59th			440		410	
66th			380	Ill	290	Ill Not using hind legs
73rd			350	Chloroformed P M Hæmorrhages round hind knee joints and suprarenals	280	Chloroformed P M Slight hæmorrhages round hind joints Small intestines hæmorrhagic

GROUP 3(*b*)—RED BANANA PULP

1st	400		300		400	
3rd	400		310		400	
8th	380		320		400	
15th	400		330		410	
22nd	440		370		460	
30th	470		380		490	
37th	450		380		500	

APPENDIX No 1—*contd*

GROUP 3(*b*)—RED BANANA PULP—*concld*

Day of experiment	BASAL DIET WITH 5 G		BASAL DIET WITH 10 G		BASAL DIET WITH 15 G	
	Weight	Remarks	Weight	Remarks	Weight	Remarks
44th	390	Showing signs of paralysis of hind legs	340		510	
51st	370		330		530	
58th	350		350		550	
65th	330		350		500	
72nd	310	Chloroformed P M Hæmorrhages round *hind joints, intestines*, kidneys and suprarenals	320	Chloroformed P M No signs of scurvy	480	Released in good health

GROUP 4—TOMATO JUICE

Day of experiment	BASAL DIET WITH 3 C C		BASAL DIET WITH 5 C C		BASAL DIET WITH 7 C C	
	Weight	Remarks	Weight	Remarks	Weight	Remarks
1st	280		500		450	
4th	300		480		480	
9th	320		520		520	
16th	350		570		530	
23rd	350		570		510	
30th	330		620		470	
37th	310		640		470	
43rd	320		630		500	
46th	300	Face ache	640		510	
54th	260	Disinclination to use hind legs *Died* P M Hæmorrhages round hind joints and in suprarenals	650		490	

APPENDIX No 1—*concld*

GROUP 4 —TOMATO JUICE—*concld*

Day of experiment	BASAL DIET WITH 3 C C		BASAL DIET WITH 5 C C		BASAL DIET WITH 7 C C	
	Weight	Remarks	Weight	Remarks	Weight	Remarks
59th	200		650		510	
65th			650		530	
70th			650	Released in good health, gained 150 gs in weight	540	Released in good health, gained 90 gs in weight

GROUP 5 —MANGO PULP

Day of experiment	BASAL DIET WITH 5 G		BASAL DIET WITH 10 G		BASAL DIET WITH 15 G		BASAL DIET WITH 20 G	
	Weight	Remarks	Weight	Remarks	Weight	Remarks	Weight	Remarks
1st	400		340		280		360	
5th	400		350		260		390	
10th	420		340		290		390	
15th	430		360		310		400	
20th	440		380		330		420	
30th	490		430		360		450	
40th	510		485		380		480	
50th	520		505		400		500	
60th	540		560		480		570	
65th	460	Signs of paralysis, hind legs Ruffled fur, loss of appetite	530		480		550	
70th	450		520		520		580	
74th	445	Chloroformed P M Hæmorrhages round hind joints Intestines injected	520	Released in good health, gain in weight 180 gs	520	Released in good health, gain in weight 240 gs	580	Released in good health, gain in weight 220 gs

APPENDIX No 2

THE EVALUATION OF VITAMIN C CONTENT

Names of articles	Amount required for adult human being, the article in question being the only source of vitamin C
FRUITS	
Bananas, yellow	50 g
Do green	Very poor, 150 g inadequate
Do red	100 g
Lime juice	50 c c
Orange juice	100 c c
Pumelo	10 c c
Pears	Very poor, 70 g merely delay the onset of scurvy
Pineapples	30 g of pulp
Pomegranate juice	Very poor, up to 200 c c inadequate
Mango	100 g of the pulp
Sweet lime	Poor, 50 c c inadequate
VEGETABLES	
Melon pumpkin	100 g
Pumpkin	100 g
Vegetable marrow	150 g
Tomato	50 c c of juice
GERMINATED PULSES, ETC	
Gram	Poor, 50 g inadequate
Peas	Poor, 50 g inadequate
Mung	Good, 30 g sufficient
Urd	40 g sufficient
Lobia	50 g sufficient

ACTION OF VENOM OF THE INDIAN DABOIA (*VIPERA RUSSELLII* VEL *VIPERA ELEGANS*) ON CERTAIN PROTOZOA

BY

LIEUT-COLONEL R N CHOPRA, M A, M D (Cantab), I M S,

AND

J S CHOWHAN, M B, B S

(*From the Department of Pharmacology, Calcutta School of Tropical Medicine and Hygiene*)

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SINCE the days of Spellanzani the paramœcia has been used as a delicate biological indicator for experimental work It is a unicellular organism whose sensitiveness and reaction to changes in the environment can be very easily observed Hamilton (1904) and Tunnicliff (1929) used this ciliate for the study of the actions of toxins and sera It has been suggested by various workers that these organisms can be used for standardizing the potency of drugs Macht and Fisher (1917-18) showed that while the alkaloids of morphine groups had no effect on the paramœcia those of papaverin groups were highly toxic Philpott (1929) worked out the minimum lethal dose of different venoms on the *Paramœcium caudatum*

In a previous paper (1931) the present authors showed that the venom of the Indian cobra (*Naia naia* vel *tripudians*) has a markedly toxic action on some of the protozoal organisms and particularly the *P caudatum* While studying the pharmacological action of the venom of the Indian Daboia—*Vipera Russellii* vel *Vipera elegans*—its effects on this unicellular organism were also studied It has been suggested by various workers that whilst the venom of the Colubrida has a toxic action on the central nervous system and particularly on the respiratory centre,

the venom of Viperidæ acts mainly locally and its action is chiefly on the vascular system The mode of death after viper bites is said to resemble that produced by histamine shock Macdonald (1922) working on *Balantidium coli* and *B suis* showed that these organisms possess a rudimentary neuro-motor apparatus which controls and co-ordinates their movements Paralysis of this mechanism produces loss of motility and death of the protozoa When testing the action of cobra venom on *P caudatum* we found evidence of this action on the neuro-muscular apparatus of this ciliate Soon after exposure to the venom, the movements of the protozoon were slowed and later were completely paralysed, its body then swelled up and disintegrated The effect of the venom of Russell's Viper was also studied on this protozoon in order to get an idea as to the fundamental differences in the action of colubrine and viperine venoms as there are no vestiges of a vascular system in this organism

It has been pointed out in a previous paper that normal saline solutions (0 9 per cent) are not suitable for protozoal work as the organisms die in it Their movements are immediately stopped and the cells shrink and break up into small fragments Balbiani (1904) pointed out that paramœcia are only isotonic with a 0·3 per cent solution of common salt We tested the vitality of these protozoa by mixing equal volumes of the culture of *Paramœcium caudatum* with saline solutions containing 0 9, 0 8, 0 7 and 0 2 per cent of sodium chloride We observed that with solutions of over 0 4 per cent strength the paramœcia died in 2 to 9 minutes To avoid this, the culture and all the dilutions were made with ordinary tap-water which has no deleterious effect on the organism

Fresh solutions of the venom were prepared daily for the experimental work Any solution which was more than one day old was discarded because in this the venom settles down at the bottom of the tube and the results obtained are inconsistent Various concentrations of the venom ranging from 1 in 1,000 and more were prepared by vigorously shaking the venom with water The solutions were turbid and at times frothy, and it took some time before they cleared

The reaction of tap-water in Calcutta varies between pH 7 0 to pH 7 4 and this variation in the hydrogen-ion concentration is not deleterious to the growth and activity of this ciliate The concentrations of the venom varying from 1 in 1,000 to 1 in 50,000 were prepared by dilution with tap-water and equal amounts of these solutions and the culture were taken, well mixed and kept in small test-tubes The resultant concentrations were labelled from 1 in 2,000 to 1 in 100,000 A drop of this mixture was examined from time to time in a hollow ground slide under the low power of a microscope and the results recorded A mixture of equal amounts of the culture and tap-water was kept as a control under similar conditions and environments in every experiment The culture of paramœcium used was one month old so that the organisms were well developed, active and mature The pH of this culture varied between 7 2 to 7 4

The effect of the Daboia venom on the Paramœcium caudatum

When equal amounts of the culture and the venom in the different concentrations are mixed and examined under the microscope the phenomenon known as the 'avoiding reaction' is at once observed It is noted that the paramœcia at first become very active in their movements and try to get away from the unfavourable surroundings The spindle and the corkscrew movement is observed Later their movements are distinctly slowed and the paramœcia move towards the periphery of the hollow ground slide This is probably due to the fact that as the depth at the margin of the hollow ground slide is shallow the concentration of the venom is less there, or perhaps in their efforts to avoid the unpalatable media, the protozoa try to get away from it and move towards the periphery and since they cannot go any further than the edge of the well they collect together there The general effect of cobra venom was to slow the movements and eventually to paralyse them The paramœcia at first moved sluggishly and later were completely paralysed, became swollen, rounded and the vacuoles became more prominent and finally the body disintegrated The venom of Russell's Viper, although it slows the movements to a certain extent, does not paralyse them as is the case with cobra venom and the protozoa do not die Even after 24 hours exposure, very few paramœcia died while others were alive though their movements were slower than normal

It has been remarked already that the venom forms a very turbid solution in high concentrations and the venom solutions when mixed with the culture form a fine precipitate In such concentrations as 1 in 200 to 1 in 2,000 the paramœcia were seen moving about in the meshes of the precipitate The only effect observed in such concentrations was that the paramœcia moved less rapidly, frequently turned on their long axis and eventually crawled at the bottom of the well Later they conglomerated together in large colonies in the meshes of the precipitate They swelled up slightly and their vacuoles became more visible and they showed a tendency to become motionless There were no signs of paralysis when they were moved by agitating the slide, but they settled down again in a seemingly lethargic condition Hamilton (1904) suggested that this slowing down of the paramœcia, when in toxic substrate, is due to the formation of long viscous threads of excretory matter at the caudal extremity These threads impede the progress and cause the paramœcia to make irregular movements in effort to get rid of it The paramœcia also become agglutinated together by means of these filaments No such filaments were observed in our experiments

A perusal of Table I shows that in concentrations varying from 1 in 200 to 1 in 2,000 the organism lived for over 24 hours in much the same way as in the control With lower concentrations varying from 1 in 4,000 to 1 in 100,000 the 'avoiding reaction' was more marked and they collected at the periphery They were seen living after 24 to 48 hours

TABLE I

Effect of Dabora venom on Paramœcium caudatum *at different dilutions*

Number	Final dilutions of venom	Effect of protozoa	REMARKS
1	Control	No effect	Equal volume of culture and tap water mixed
2	1 in 200	Alive after 24 hours	Equal volume of culture and venom Flocculent precipitate, the paramœcia sank to the bottom and collected in colonies Movements slowed and later paralysed
3	1 in 400	Do	
4	1 in 600	Do	
5	1 in 800	Do	
6	1 in 2,000	Alive after 48 hours	Mixture turbid, 'avoiding reaction', movements slowed, collected at the periphery Swollen, move on agitation
7	1 in 4,000	Do	
8	1 in 6,000	Do	
9	1 in 8,000	Do	
10	1 in 10,000	Do	
11	1 in 25,000	Do	
12	1 in 50,000	Do	
13	1 in 100,000	Do	

The effect of change of hydrogen-ion concentration

Bodin (1921) showed that the pH of the paramœcium culture alters with age and Dale and Lovatt Evans pointed out that the vitality of the protozoa is changed with increase of hydrogen-ion concentration The rate of locomotion increases with the decrease of pH and vice versa Aurin Chase and Glaser (1930) showed that with any alteration in the hydrogen-ion concentration towards acid or alkali there ... st an increase in the movements which is soon followed by a rapid ... mixture w... n return to normal After the period of recovery the speed is main- low power of a m... brium This recovery and return of speed to equilibrium depends the culture and ta... acid or alkali is organic or inorganic If the acid is CO_2 or an ments in every ex... as valeric acid, the rate of equilibrium is more than that at pH 7 old so that the orga... an inorganic acid It was shown that carbonic acid or valeric culture varied betw... ramœcia more readily than an inorganic mineral acid, since the

former penetrated the protozoal cells more completely Crane (1921) stated that the paramœcia can live for twenty-four hours in any pH varying from 7 6 to 5 In our experiments we observed that this was only true if the pH was reduced by CO_2 and if other mineral acids such as HCl, H_2SO_4, etc , were used the paramœcia died immediately probably due to the toxic effects of the dissociated ions of these acids in the substrate

A perusal of Table II will show that when hydrogen-ion concentration of the culture was raised with N/10 NaOH to pH 8, the paramœcia became more vulnerable to the venom of Russell's Viper They died in about 1 to 2 hours in concentrations of 1 in 200 and 1 in 100 , with higher dilutions from 1 in 600 to 1 in 800 they died in 24 hours , still higher dilutions did not appear to have any effect within 24 to 48 hours A perusal of Table III will show the effect of the viper venom when the hydrogen-ion concentration of the culture was lowered by bubbling CO_2 as far as pH 5 It will be seen that the venom had no effect on the paramœcium in acid media, the only remarkable change observed being that the granules became more prominent at the caudal end

TABLE II

Effect of Viper venom on Paramœcium caudatum *at pH above* 7 0

Number	Final dilutions of venom	Effect on paramœcium at different pH raised by N/10 NaOH		Remarks
		pH 8 0	pH 7 0	
1	Control	No effect	Alive after 24 hours	
2	1 in 200	Death in 1 25 hours	Do	
3	1 in 400	Do	Do	
4	1 in 600	Death within 24 hours	Do	
5	1 in 800	Do	Do	
6	1 in 2,000	Alive after 24 hours	Do	
7	1 in 4,000	Do	Do	
8	1 in 6,000	Do	Do	
9	1 in 8,000	Do	Do	
10	1 in 10,000	Do	Do	
11	1 in 25,000	Do	Do	
12	1 in 50,000	Do	Do	
13	1 in 100,000	Do	Do	

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STANDARDS FOR PREDICTING THE NORMAL VITAL CAPACITY OF THE LUNGS IN SOUTH INDIAN WOMEN FROM HEIGHT, WEIGHT AND SURFACE AREA *

BY

ELEANOR D MASON,

Women's Christian College, Madras

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In a previous paper (Mason, 1930) a statistical analysis was made of the measured vital capacity of 266 young South Indian women and of the relation between this measurement and height, weight and body surface area Of these three functions height was found to have the highest correlation with vital capacity and from the data a regression equation was derived and a curve plotted from which the average vital capacity of a South Indian woman could easily be determined from measured height The results were closely compared with a similar analysis of the vital capacities of young American women (Turner, 1927) and showed a mean vital capacity 22 per cent less than the American value for women of the same height as the Indian mean height

The present study is based on 587 new measurements on South Indian women, which are analysed separately and together with the original 266 The subjects were students and members of staff in four women's colleges and two medical schools in Madras and Vellore, together with a much smaller number of nurses, school teachers and school girls of 17 years or more They represent a variety of South Indian races—Tamils, Malayalis, Telugus, Kanarese, Konkanis and Coorgs The age range was 16 to 35 but the great majority were from 17 to 22 years

The apparatus used was the same as for the first series, the Collins spirometer,† found by Turner, after a study of several types of spirometers used in American

* A paper read at the Section of Medical and Veterinary Research, Indian Science Congress, Bangalore, January 1932

† Manufactured by Warren E Collins, 555, Huntington Avenue, Boston, Mass, U S A

women's colleges, to be thoroughly good, filling all the requirements for getting accurate measurements namely 'a light bell, accurately counterpoised, and moving without friction so that it starts easily and shows no back pressure at the end', and 'a mouthpiece and connecting tubing of sufficient aperture so that the air can be received by the machine at any speed which the subject finds easy to use' At the time of measurement the experimenter watched each student closely as she blew into the spirometer to detect error in technique or effort which would fail to give the maximum value Those who did not perform correctly were given more than three trials, but in the great majority of cases the highest value reached in three trials was recorded Heights were measured barefoot and weights were taken with clothing and corrected for the type of clothing worn Surface areas were calculated from the Du Bois chart based on the Du Bois height-weight formula [Fig 1 (Du Bois, 1916)]

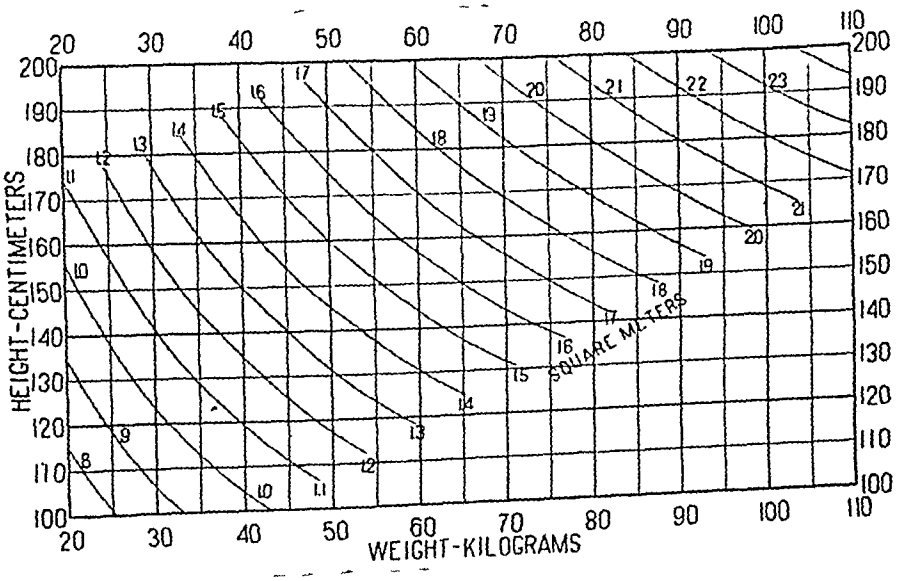

Fig. 1 —The Du Bois chart for estimating surface area from measured height and weight according to the formula Area (sq m)$=Wt^{0.4-}\times Ht^{0.7-5}\times 71\,84$

The statistical study has been made with the counsel and help of Mr W F Kibble, Professor of Mathematics at the Madras Christian College Sheppard's correction for grouping was used in calculating the standard deviations

Results

Tables I to III show the original data from which the calculations were made Table IV shows the means, standard deviations, and coefficients of variation

of heights, weights, surface areas and vital capacities, and the coefficients of correlation of the vital capacities with each of the other three measurements The American standards given for comparison are taken from a second paper by Turner (1930*a*) in which she shows that the vital capacities recorded in her earlier paper, with which the first South Indian measurements were compared, were too low, averaging 2 99 litres as compared with 3 28 in the second series The increase she attributes to an 'increase in accuracy' of measurement due to the ease with which the Collins spirometer is blown The apparatus used in the earlier series was as good as was available at the time the measurements were made

TABLE I

Correlation between vital capacity and height in South Indian women

Vital Capacity in litres	Height in cm											
	Total	130—134 5	135—139 5	140—144 5	145—149 5	150—154 5	155—159 5	160—164 5	165—169 5	170—174 5	175—179 5	180—184 5
Total	853	1	13	80	203	313	169	58	12	2	1	1
1 05—1 34	4			1	3							
1 35—1 64	41		5	4	16	12	4					
1 65—1 94	187		2	31	64	60	23	6	1			
1 95—2 24	322		5	38	82	129	47	18	1	1		1
2 25—2 54	192	1	1	6	31	79	56	14	2	1	1	
2 55—2 84	79				6	30	26	11	6			
2 85—3 14	24				1	2	11	8	2			
3 15—3 44	4					1	2	1				

TABLE II

Correlation between vital capacity and weight in South Indian women

Vital capacity in litres	Weight in kg															
	Total	26 5—31 4	31 5—36 4	36 5—41 4	41 5—46 4	46 5—51 4	51 5—56 4	56 5—61 4	61 5—66 4	66 5—71 4	71 5—76 4	76 5—81 4	81 5—86 4	86 5—91 4	91 5—96 4	96 5—101 4
Total	853	10	97	254	252	148	55	21	11	2		2				1
1 05—1 34	4	1	2	1												
1 35—1 64	41	1	10	18	9	3		.								
1 65—1 94	187	7	38	71	48	15	7	1								
1 95—2 24 .	322	1	35	110	95	50	18	6	5	1		1				
2 25—2 54	192	.	10	44	66	43	20	4	4	1						
2 55—2 84 .	79	.	2	9	26	26	7	6	1			1	.			1
2 85—3 14 .	24	.	..	1	8	10	3	2	.							
3 15—3 44 .	4	.	.	.	.	1		2	1							

The results obtained from the larger number of subjects in the second series of Indians (Table IV, Column II) vary but slightly from those in the first series The agreement is close enough to justify the use of the combined data in deriving

TABLE III

Correlation between vital capacity and surface area in South Indian women

Vital capacity in litres	Surface area in sq m											
	Total	1·0—1·09	1·1—1·19	1·2—1·29	1·3—1·39	1·4—1·49	1·5—1·59	1·6—1·69	1·7—1·79	1·8—1·89	1·9—1·99	2·0—2·09
Total	853	2	40	211	302	206	68	20	2	1		1
1·05—1·34	4		2	2								
1·35—1·64	41		5	15	18	3						
1·65—1·94	187	1	23	64	70	24	5					
1·95—2·24	322	1	7	100	120	62	23	7	2			
2·25—2·54	192		3	25	72	68	20	4				
2·55—2·84	79			5	20	34	13	5		1		1
2·85—3·14	24				2	14	6	2				
3·15—3·44	4					1	1	2				

prediction standards The correlation between vital capacity and height is not so high as in the first series but the correlations between vital capacity and surface area and especially vital capacity and weight are higher Distinctly the best of the correlations is that with surface area but whether this is a true correlation cannot be established until actual measurements are made of the surface area of Indians There is conflicting evidence on the applicability of the Du Bois formulæ (1915 and 1916) to the surface area of the Chinese (Stevenson, 1928) Mason and Benedict (1931) have noted that in 54 South Indian women used as subjects for metabolism studies the trunk length as measured by the sitting height is shorter and the legs

longer in proportion to standing height than is normal for westerners and it may be that other measurements are sufficiently different to require new surface area formulæ for Indians

TABLE IV

Statistical data regarding two series of South Indian women totalling 853, *and* 1,337 *American women*

	South Indian women			American women
	Series I	Series II	Series I and II	
Number tested	266	587	853	1,337
Means —				
Height, cm	152 54	151 54	151 86	162 83
Weight, kg	43 82	43 40	43 49	55 50
Surface area, sq m	1 37	1 35	1 36	1 58
Vital capacity, litres	2 134	2 160	2 150	3 28
Standard deviations —				
Height	5 60	5 95	5 88	5 9965
Weight	6 45	7 17	6 96	7 1980
Surface area	0 11	0 11	0 11	0 1123
Vital capacity	0 350	0 331	0 337	0 4384
Coefficients of variation —				
Height	0 0367	0 0393	0 0387	0 0369
Weight	0 1474	0 1652	0 1602	0 1297
Surface area	0 0782	0 0815	0 0822	0 0711
Vital capacity	0 1640	0 1528	0 1567	0 1329
Coefficients of correlation —				
Vital capacity, height	0 464 ± 0 032	0 418 ± 0 023	0 419 ± 0 019	0 4958
Vital capacity, weight	0 270 ± 0 038	0 408 ± 0 023	0 407 ± 0 019	0 3754
Vital capacity, surface area	0 424 ± 0 034	0 479 ± 0 021	0 470 ± 0 018	0 4870

From the combined data on South Indians (Tables I to III and Column III, Table IV) regression equations have been derived for vital capacity on height, weight and surface area These are —

Vital capacity = 0 024 height — 1 494
Vital capacity = 0 019 weight + 1 292
Vital capacity = 1 417 surface area + 0 223

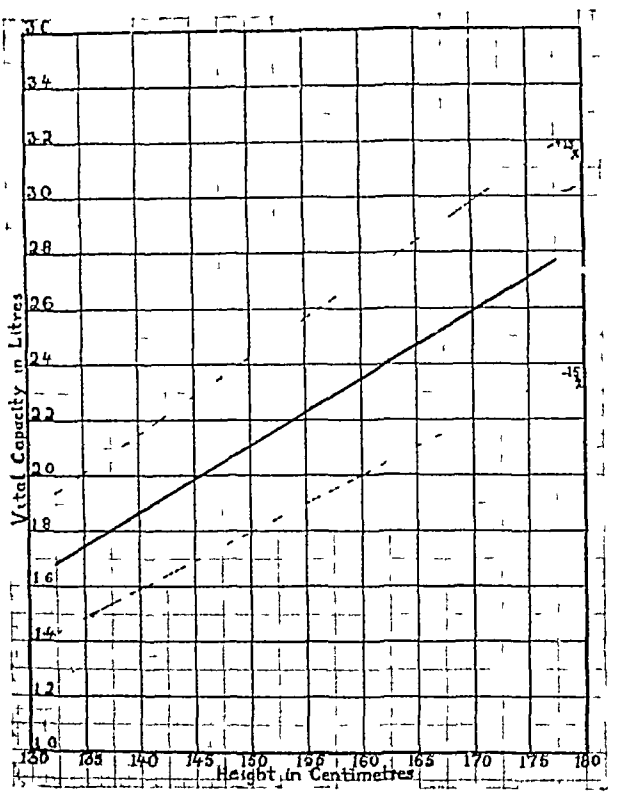

Fig 2 —Chart for finding the average vital capacity for young adult South Indian women from measured height The solid line is the line of average vital capacity for the heights given at the bottom of the chart Vital capacity measurements falling above the +15 per cent line and below the —15 per cent line represent very high and very low vital capacities

In Figs 2, 3 and 4 are shown the lines of regression plotted from these equations, and from these charts the mean vital capacity can readily be determined for any

measured height or weight or calculated surface area In addition, lines representing plus and minus 15 per cent of the mean values are shown The great majority of measured vital capacities will fall between these two lines, those falling outside being very high and very low If Myer's statement that vital

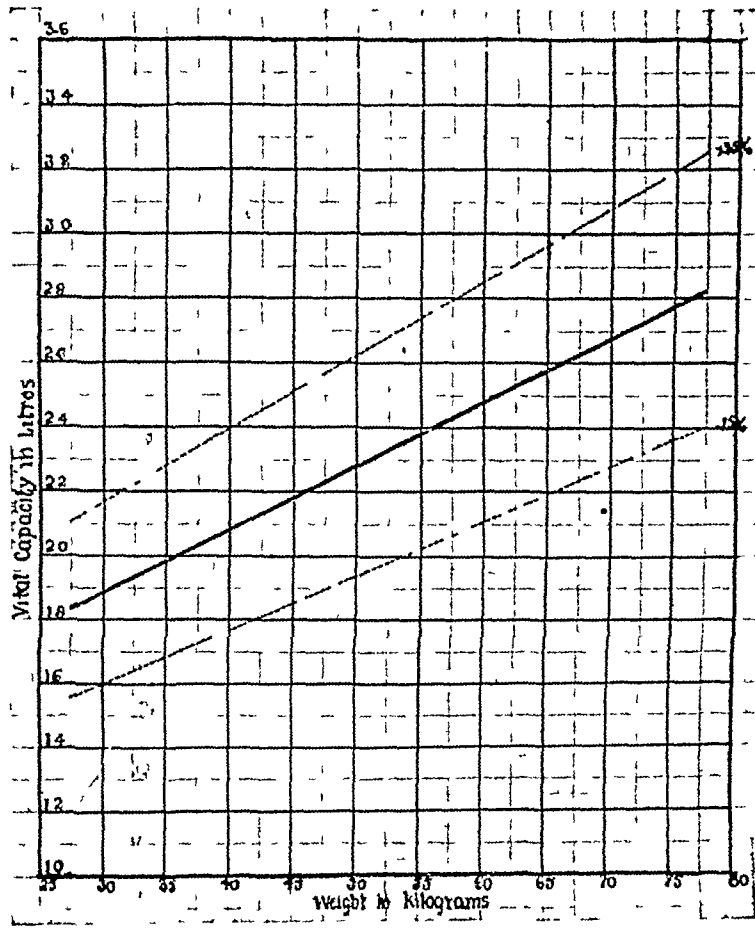

Fig 3—Chart for finding the average vital capacity for young adult South Indian women from measured weight

Lines as in Fig 2

capacities 15 per cent or more below normal should lead one to suspect pulmonary or cardiac disease be correct for Indians, then the —15 per cent line should be of value to institutions which require routine physical examinations and to clinicians The evidence of clinicians is needed for establishing the danger zone of low vital capacities for Indians

A study has also been made of the three largest race groups represented among the subjects studied, 389 Tamils, 291 Malayalis and 129 Telugus The data and results for these three groups are shown in Tables V to XI The number of Telugus is too small for statistical treatment, but it is interesting to note that the upper

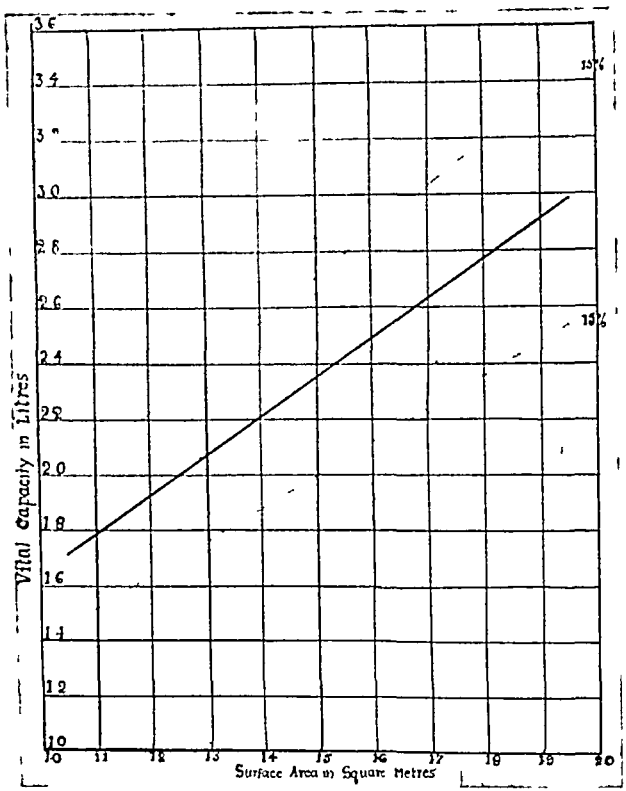

Fig 4 —Chart for finding the average vital capacity for young adult South Indian women from calculated surface area Surface area is derived from the Du Bois height weight formula and chart

Lines as in Fig 2

extreme of all three measurements falls in this group, in different individuals, and that in spite of the fact that the extraordinary weight of 101 kg and the very high vital capacity of 3 41 litres occur in this group, the Telugus are still below the average in weight and distinctly below the average in vital capacity It is hoped that someone in the Telugu district will collect quantitative measurements and determine whether the Tamil standards will need to be lowered for Telugus The higher value of the Malayali vital capacity is noteworthy This may be a racial

difference apart from activity, but it seems more likely that it is related to the fact that almost all Malayali women live more in the open than Tamil women and that they swim from the time they are children Fig 5 shows for comparison the lines of regression of vital capacity on height for the two largest race groups, and of

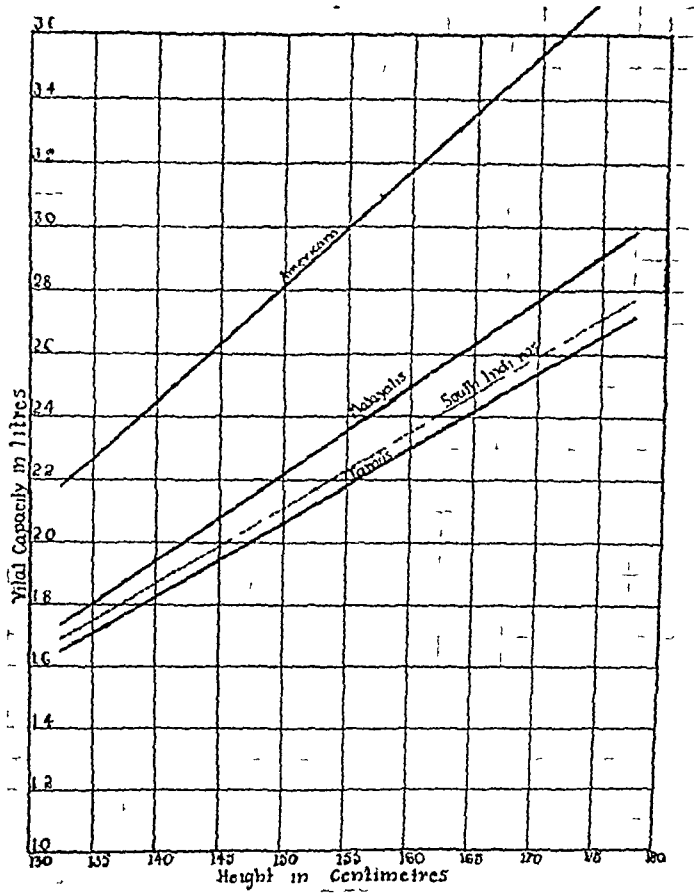

Fig 5—Chart comparing the average vital capacity for measured height for Tamil, Malayali and American women The dotted line shows the average for combined groups of South Indian women as in Fig 2

American women, and Figs 6 and 7, vital capacity and weight on vital capacity on surface area for the same groups The equations for these are —

Tamils	Malayalis	Americans
V C = 0 023 Ht − 1 455	V C = 0 028 Ht − 1 981	V C = 0 036 Ht − 2 706
V C = 0 020 Wt + 1 240	V C = 0 021 Wt + 0 907	V C = 0 023 Wt + 2 017
V C = 1 393 S A + 0 211	V C = 1 505 S A + 0 186	V C = 1 901 S A + 0 279

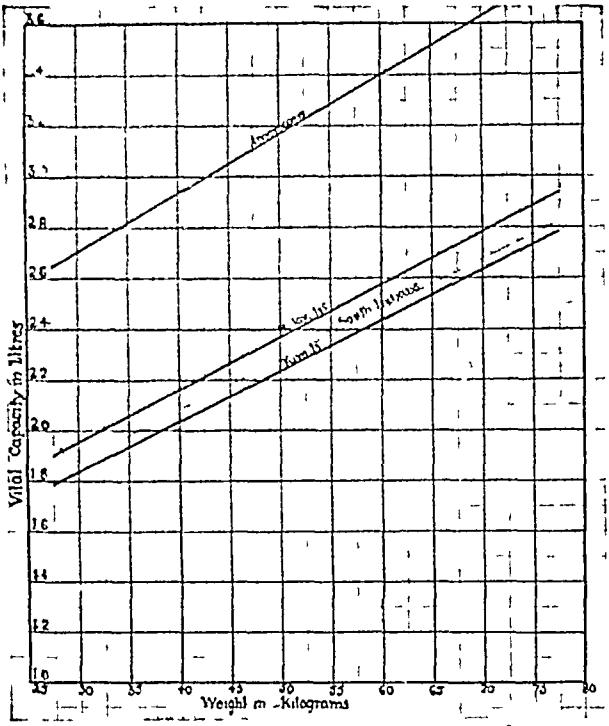

Fig 6—Chart comparing the average vital capacity for measured weight for Tamil, Malayali and American women The dotted line shows the average for combined groups of South Indian women as in Fig 3

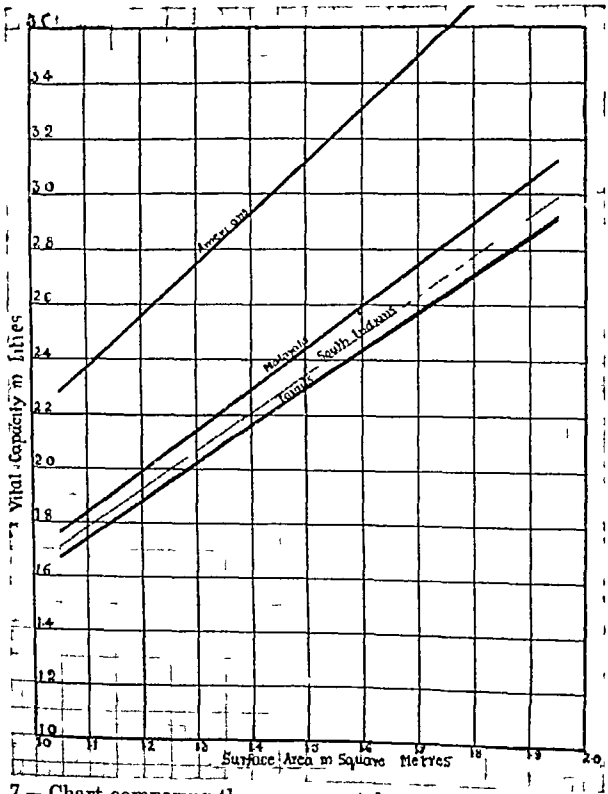

Fig 7—Chart comparing the average vital capacity for calculated surface area for Tamil, Malayali and American women The dotted line shows the average for combined groups of South Indian women as in Fig 4

TABLE V.

Correlation between vital capacity and height in Tamil women

Vital capacity in litres	Total	Height in cm									
		130—134 5	135—139 5	140—144 5	145—149 5	150—154 5	155—159 5	160—164 5	165—169 5	170—174 5	175—179 5
Total	389		8	45	91	133	77	27	6	1	1
1 05—1 34	1			1							
1 35—1 64	25		3	4	9	9					
1 65—1 94	95		1	17	35	27	12	3			
1 95—2 24	160		3	21	39	56	32	9			
2 25—2 54	78		1	2	7	30	25	9	2	1	1
2 55—2 84	23					10	6	5	2		
2 85—3 14	6				1	1	1	1	2		
3 15—3 44	1						1				

TABLE VI

Correlation between vital capacity and height in Malayali women

Vital capacity in litres	Total	Height in cm								
		130—134 5	135—139 5	140—144 5	145—149 5	150—154 5	155—159 5	160—164 5	165—169 5	170—174 5
Total	291	1	3	20	76	108	58	21	3	1
1 05—1 34	1				1					
1 35—1 64	6		1		4		1			
1 65—1 94	50			9	17	16	5	2	1	
1 95—2 24	101		2	9	33	42	7	7		1
2 25—2 54	76	1		2	15	37	20	1		
2 55—2 84	39				6	12	15	4	2	
2 85—3 14	16					1	9	6		
3 15—3 44	2						1	1		

TABLE VII

Correlation between vital capacity and weight in Tamil women

Vital capacity in litres	Weight in kg									
	Total	26 5—31 4	31 5—36 4	36 5—41 4	41 5—46 4	46 5—51 4	51 5—56 4	56 5—61 4	61 5—66 4	66 5—71 4
Total	389	4	51	121	114	66	18	10	3	2
1 05—1 34	1		1							
1 35—1 64	25		8	8	6	3				
1 65—1 94	95	3	22	32	25	9	4			
1 95—2 24	160	1	19	56	50	26	3	3	1	1
2 25—2 54	78			21	27	16	8	4	1	1
2 55—2 84	23		1	3	6	8	3	2		
2 85—3 14	6			1		4		1		
3 15—3 44	1								1	

TABLE VIII

Correlation between vital capacity and weight in Malayali women

Vital capacity in litres	Weight in kg											
	Total	26 5—31 4	31 5—36 4	36 5—41 4	41 5—46 4	46 5—51 4	51 5—56 4	56 5—61 4	61 5—66 4	66 5—71 4	71 5—76 4	76 5—81 4
Total	291	3	29	77	90	56	23	7	5			1
1 05—1 34	1			1								
1 35—1 64	6	1	1	4								
1 65—1 94	50	2	9	20	15	2	1	1				
1 95—2 24	101		10	36	26	17	7	2	2			1
2 25—2 54	76		8	15	24	17	10		2			
2 55—2 84	39		1	1	18	15	2	1	1			
2 85—3 14	16				7	5	3	1				
3 15—3 44	2							2				

TABLE IX

Correlation between vital capacity and surface area in Tamil women

Vital capacity in litres	Surface area in sq m								
	Total	1 0—1 09	1 1—1 19	1 2—1 29	1 3—1 39	1 4—1 49	1 5—1 59	1 6—1 69	1 7—1 79
Total	389	1	24	98	138	91	30	6	1
1 05—1 34	1		1						
1 35—1 64	25		4	8	11	2			
1 65—1 94	95		14	35	28	15	3		
1 95—2 24	160	1	4	47	65	32	8	2	1
2 25—2 54	78		1	5	31	28	12	1	
2 55—2 84	23			3	2	12	5	1	
2 85—3 14	6				1	2	2	1	
3 15—3 44	1							1	

TABLE X

Correlation between vital capacity and surface area in Malayali women

Vital capacity in litres	Surface area in sq m								
	Total	1 0—1 09	1 1—1 19	1 2—1 29	1 3—1 39	1 4—1 49	1 5—1 59	1 6—1 69	1 7—1 79
Total	291		10	71	96	80	25	8	1
1 05—1 34	1			1					
1 35—1 64	6		1	3	1	1			
1 65—1 94	50		6	14	23	6	1		
1 95—2 24	101		2	35	35	18	8	2	1
2 25—2 54	76		1	16	25	26	6	2	
2 55—2 84	39			2	11	19	5	2	
2 85—3 14	16				1	10	4	1	
3 15—3 44	2						1	1	

TABLE XI

Statistical data comparing three racial groups of South Indian women

	Tamils	Malayalis	Telugus
Number tested	389	291	129
Means —			
Height, cm	151 64	151 97	151 86
Weight, kg	42 91	44 05	43 17
Surface area, sq m	1 35	1 37	1 36
Vital capacity, litres	2 093	2 244	2 055
Standard deviations —			
Height	6 13	5 55	5 95
Weight	6 40	6 78	8 16
Surface area	0 11	0 11	0 12
Vital capacity	0 309	0 349	[0 323
Coefficients of variation —			
Height	0 0404	0 0365	0 0392
Weight	0 1493	0 1540	0 1891
Surface area	0 0793	0 0789	0 0899
Vital capacity	0 1476	0 1556	0 1574
Coefficients of correlation —			
Vital capacity, height	0 464 ± 0 027	0 441 ± 0 032	
Vital capacity, weight	0 412 ± 0 028	0 400 ± 0 033	
Vital capacity, surface area	0 483 ± 0 026	0 466 ± 0 031	

The difference between the western group and the Indian groups is very striking Expressed in the ratios introduced by West (1920) the following values are found for Indian, Chinese (Foster and Hsieh, 1923) and American women —

	Indians	Chinese	Americans
Number tested	853	75	1,337
$\frac{\text{Vital capacity, c c}}{\text{Height, cm}}$	14 2	14 0	20 1
$\frac{\text{Vital capacity, litres}}{\text{Surface area, sq m}}$	1 58	1 50	2 07

Discussion

The significance of the vital capacity measurement —The studies of Turner (1930*b*) on normal women and of many workers on pathological cases show a close relation between vital capacity and health The influence of disease upon vital capacity is discussed by Myers (1925) in his book, 'The Vital Capacity of the Lungs' This influence may be summarized as follows past diseases which leave lowered vital capacities are those affecting the thorax, especially pleurisy, tuberculosis and cardiac disease, present diseases where reduced vital capacity measurements have clinical significance are cardiac disease, pleurisy, pneumonia, pulmonary tuberculosis and to a lesser extent hyperthyroidism, emphysema, asthma, pneumothorax, pulmonary abscess, and new growths The use of the vital capacity measurement in the diagnosis and more particularly in the prognosis and subsequent treatment of these diseases has been found to be very helpful

Turner (1930*b*) has made a very interesting study of normal women, i e , college students, of high and low vital capacities, and finds the vital capacity measurement a useful index of general physical fitness She finds the students of high vital capacity superior in vigor, in athletics, in general health and even in academic work She has also studied the question of the fixity of the vital capacity level in the same individuals Measurements of students taken at the end of four years of college life and compared with those taken when they first entered college show no appreciable change, thus justifying the use of her standards based on 1st year college women for adult women in general On the other hand, when a group of very low vital capacity students was placed under the special direction of the physical training department and given for nine months special exercises for thoracic development and mobility, the increase in vital capacity in 50 per cent of the cases was 210 to over 600 c c Her evidence from the past history of high and low vital capacity students points to the years of adolescence as the most effective time for training in thoracic development A good vital capacity attained in these years

does not appear to be easily lost even when subsequent years include very little sturdy exercise

It is hoped that similar experiments to see whether the vital capacity of very low Indian students can be raised by special training can be made in the Women's Christian College A study is also being made of the effect of four years of normal college life with regular out-door exercise on the general level of the student vital capacity

Race and vital capacity —It seems obvious that some of the difference between the vital capacities of Indian and American women is due to difference in activity, particularly activity during childhood and adolescence, and that as India lays increasing emphasis on physical training in schools and colleges and Indian women and girls achieve increasing freedom of movement this difference will be diminished But that Indian women whose vital capacity for their mean height of 151 6 cm is now 2 15 litres, will ever, through any amount of physical training, attain the American level for the same height of 2 84 litres seems most unlikely There appears to be here, and in the findings of Bhatia (1929) for Indian men*, a real racial difference How much of this difference may be strictly anatomical could be determined by a series of external anthropological measurements and by a study of the weight of the lungs Baron von Eickstedt, Director of the Anthropological Institute of the University of Breslau, who has made an anthropological study of thousands of South Indian peoples which did not, unfortunately, include thoracic measurements other than the shoulder-breadth, has expressed in a letter to the writer his personal opinion that the difference will prove to be physiological and not anatomical The fact that very low vital capacities are also found in the Chinese (Foster and Hsieh, 1923) whose physical build is different from that of the Indian would suggest that a low vital capacity is a physiological characteristic of at least some Oriental peoples rather than an anatomical variable

Summary

Measurements of heights, weights and vital capacity of 587 new South Indian women subjects are shown to have no significant variation from the measurements

* Since this paper went to press a paper has appeared on 'The Vital Capacity of 103 Male Medical Students in South India' (Krishnan, B T, and Vareed, C, *Ind Jour Med Res*, **19**, No 4, p 1165) The average vital capacity found is 2 93 litres If the Anglo Indian students, of whom a separate study should be made, are omitted, the average becomes 2 86 litres, which is only 67 per cent of the average for British and American men of the same height, as compared with 76 per cent reported for South Indian women in the present paper Bhatia s average for 100 male medical students in Bombay (presumably Marathis) is 3 091 litres which is 73 per cent of the average for western men of the same height It seems unlikely that the South Indian men are as low as reported by Krishnan and Vareed Since it is impossible to get too *high* a vital capacity measurement and very easy to get one too low, it would be worth while to check and extend the series on South Indian men with a better type of spirometer

on 266 women recorded in an earlier paper From the combined data regression equations are derived for vital capacity on height, weight and surface area and charts drawn for predicting normal vital capacities from these three measurements

Analysis of three race groups, Tamils, Malayalis and Telugus, shows that the Malayalis have a higher vital capacity Comparative charts are drawn for Tamil, Malayali and American women The average vital capacity for Indian women is found to be 76 per cent of the average for American women of the same height as the Indian mean height

The significance of the vital capacity measurement in health and disease and the question of racial influence on vital capacity are discussed

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THE RELATIVE SUSCEPTIBILITY OF CHINESE HAMSTERS (*CRICETULUS GRISEUS*) TO KALA-AZAR BY THE ORAL AND SUBCUTANEOUS ROUTES

BY

LIEUT.-COLONEL H. E. SHORTT, F.R.S., I.M.S.,

AND

C. S. SWAMINATH

(*From the Trypanosomidæ Inquiry, Pasteur Institute of India, Kasauli*)

[Received for publication, March 7, 1932]

OUR experience with hamsters had shown that a percentage approaching 100 per cent became infected with kala-azar when the infective material, whether flagellate or in the Leishman-Donovan body form, was introduced by the intraperitoneal route. As infection by this route is not possible under natural conditions it becomes important to decide which of the two most probable natural routes of infection, viz., the subcutaneous (intracutaneous) or oral, gives the highest number of infections under experimental conditions.

A study of the work done on this subject shows some discrepancies in the results of different workers and, as comparative experiments in no case appear to have been done on a large scale, we thought it advisable to place on record our own findings in order that they might be considered along with those of other observers.

Young, Smyly and Brown (1926) in a series of infection experiments with Chinese hamsters and the Leishman-Donovan body form of *L. donovani* record an infection rate of 21 per cent when the parasites were introduced by the subcutaneous route as against 67 per cent when they were introduced by the intraperitoneal route, the same dose of inoculum being used. Hindle and Patton (1926) working with the same species of Chinese hamsters record entirely negative results when Leishman-Donovan body forms of *L. donovani* were introduced by the percutaneous and subcutaneous routes.

Having a number of hamsters not required for other experiments we decided to do a small series of experiments, using both the Leishman-Donovan and the flagellate forms of *L. donovani*, with a view to comparing the relative susceptibility to this parasite of the Chinese hamster, *C. griseus*, by the subcutaneous or oral routes.

Experiments

The inoculum, whether Leishman-Donovan body or cultural forms of *L donovani*, was accurately measured in a tuberculin syringe and, in each experiment, the same dose was administered subcutaneously into the loose tissues of the back or orally by dropping it slowly into the mouth of the animal

As the animals generally ejected a considerable portion of the material dropped into their mouths it should be noted that the dose administered subcutaneously, and therefore necessarily retained *in toto*, was always actually in excess of the effective dose given by the oral route thereby giving an advantage to the subcutaneous route The details of the experiments are recorded below in tabular form —

TABLE I

Showing the results of infecting Chinese hamsters with the Leishman-Donovan body form of L donovani *by the subcutaneous and oral routes respectively*

Hamsters	Infecting route	Inoculum	Duration in days and method of termination of experiment	Result of examination	
				Cultural	Direct microscopical
S L D 1	Subcutaneous	0 25 c c of liver and spleen emulsion of infected hamster	133 Animal killed	+	−
„ 2 .	Do	Do	133 Do	+	+
„ 3	Do	Do	133 Do	+	+
„ 4 .	Do	Do	133 Do	+	+
„ 5	Do	Do	133 Do	−	−
„ 6	Do	Do	119 Animal died	No culture	+
„ 7	Do	Do	133 Animal killed	+	+
„ 8	Do	Do	133 Do	+	+
„ 9	Oral	Do	133 Do	+	+
„ 10	Do	Do	133 Do	+	+
„ 11	Do	Do	133 Do	+	+
„ 12	Do	Do	133 Do	+	+
„ 13	Do	Do	133 Do	+	+
„ 14	Do	Do	133 Do	+	+
„ 15 .	Do	Do	133 Do	+	+
„ 16	Do	Do	133 Do	+	+

The results in this experiment show that out of sixteen hamsters given the same dose of the Leishman-Donovan body form of *L donovani*, half by the subcutaneous and half by the oral route, seven out of eight of the former and all of the latter became infected

TABLE II

Showing the results of infecting Chinese hamsters with the flagellate form of L donovani *by the subcutaneous and oral routes respectively*

Hamsters	Infecting route	Inoculum	Duration in days and method of termination of experiment	Result of examination	
				Cultural	Direct microscopical
S C 1	Subcutaneous	0 25 c c of centrifuged culture of *L donovani*	142 Animal killed	+	+
„ 2	Do	Do	142 Do	+	+
„ 3	Do	Do	142 Do	+	+
„ 4	Do	Do	142 Do	+	+
„ 5	Do	Do	142 Do	+	+
„ 9	Oral	Do	142 Do	−	−
„ 10	Do	Do	142 Do	+	+
„ 11	Do	Do	142 Do	−	−
„ 12	Do	Do	142 Do	−	−
„ 13	Do	Do	142 Do	+	+

The results in this experiment show that out of ten hamsters given the same dose of cultural forms of *L donovani*, half by the subcutaneous and half by the oral route, all of the former and two out of five of the latter became infected

CONCLUSIONS

1 The Chinese hamster *Cricetulus griseus* is very susceptible to infection with *L donovani* in both its flagellate and non-flagellate stages whether the infection be by the oral or subcutaneous routes

2 With the non-flagellate stage of *L donovani* there is practically an equal susceptibility by the two routes

3 With the flagellate stage of *L donovani* the susceptibility is greater by the subcutaneous route

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THE DISTRIBUTION AND CAUSE OF ENDEMIC GOITRE IN THE UNITED PROVINCES

Part II

THE NAKED EYE AND MICROSCOPIC ANATOMY OF GOITRE, WITH SPECIAL REFERENCE TO THE ENDEMIC GOITRE OF THE UNITED PROVINCES *

BY

LIEUT.-COLONEL H STOTT, MD, FRCP, DPH, IMS,
Professor of Pathology, King George's Medical College, Lucknow

[Received for publication, March 7, 1932]

IN attempting to deal with the distribution and cause of endemic goitre in the U P, it became necessary to examine the naked eye and microscopic features of the goitre specimens obtained A summary of these results is set out in the following paper —

Source of specimens

Owing to the difficulty of obtaining post-mortem material and to the reluctance of goitrous persons for an operation even if indicated, specimens of goitre from the U P endemic area were not easily obtained I have, however, succeeded in collecting some 25 specimens from police post-mortems, and through the kindness of surgeons operating in the endemic districts and at King George's Hospital Many specimens from other pathological museums in India have, too, also been examined

Pathological anatomy

Each specimen from the U P endemic area examined indicated that it commenced as the primary diffuse colloid enlargement usually found in simple non-toxic endemic goitre The gross appearance, however, varied in different goitres and in different parts of the same goitre being due to the extent of secondary

* Part I of this investigation dealing with clinical and ætiological observations, and the distribution of endemic goitre in the United Provinces was published in the *Indian Journal of Medical Research* of April 1931

changes In this U P series such varied appearances are best described under the following seven heads and with the diagrams of the text-figure, the photographs 1 to 4 (Plate VI) and microphotographs 1 to 6 (Plate VII)

I Simple hypertrophy

The first short-lived stage of a diffuse colloid goitre is probably a slight general enlargement from a compensatory hypertrophy of functioning tissue to meet an increased demand by the body for thyroxin Microscopically, such a goitre is indistinguishable from the structure of the normal gland, and the enlargement is mainly due to an increase in the number of follicles Each follicle is still approximately of the same size, allowing for the usual difference in the plane of the various tangential sections, but perhaps some small increase in the amount of the intra-follicular colloid can be made out The vesicles are still lined by a cuboidal-celled epithelium with deeply stained nuclei No specimen in this stage was observed (*see* Plate VII, microphotograph 1, *normal thyroid*)

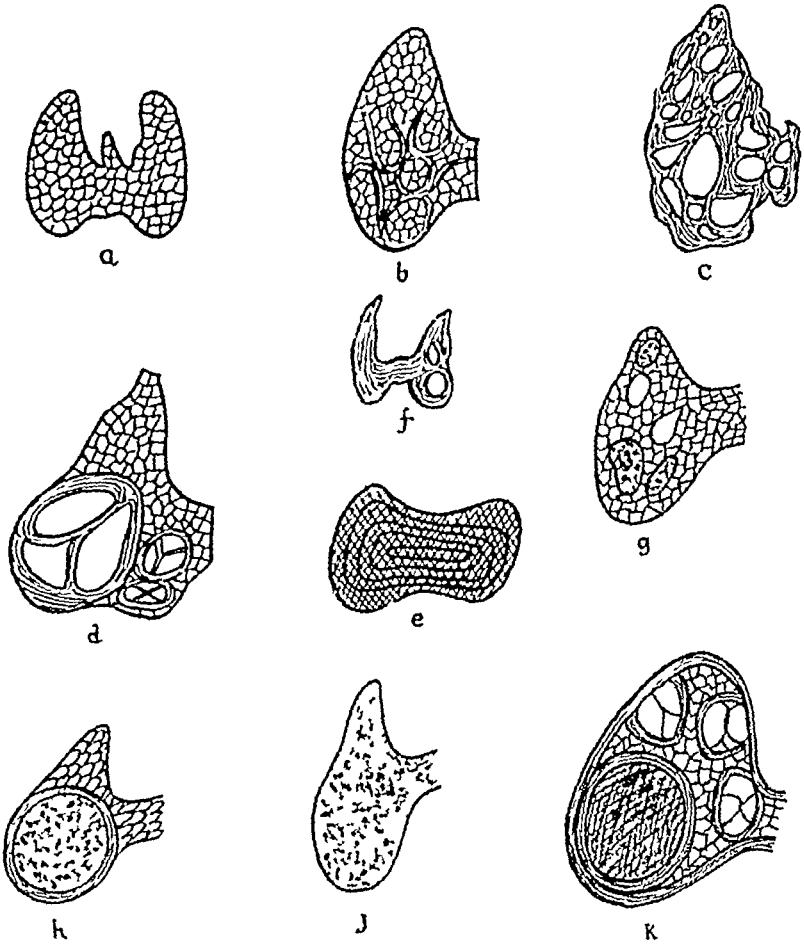
II Pressure atrophy and cystic degeneration

The second stage is one of diffuse and increasing distension of each follicle of the whole gland from colloid retention, which causes a pressure atrophy of the functioning cuboidal epithelium the cells of which become markedly flattened The thyroid enlargement is now due to colloid retention and the gland is in a condition of atrophic degeneration rather than of hypertrophy for the functional capacity of each gland unit is diminished As each follicle is affected, the enlargement is fairly uniform and bilateral The gland in this stage is elastic and smooth on palpation and, on section, shows a honeycombed structure filled with translucent amber-coloured colloid, which may be stained a reddish or brownish colour from slight hæmorrhage (Text-figure, *b*, and Plate VII, microphotograph 2)

The pressure atrophy of the epithelium and of the interfollicular tissue increases and adjoining distended follicles coalesce to form small colloid cysts This process may be distributed as multiple small colloid cysts, throughout the whole gland (Text-figure, *c*), or as a collection of small cysts in a localized area which may unite to form a large colloid cyst (Text-figure, *d*) More usually these two types are combined in varied degrees Large colloid cysts project from the surface of the gland and are readily palpated as a 'nodular' colloid goitre—which clinically becomes commoner as middle age is approached

III Fibrosis (*a*) *Perilobular or capsular, and* (*b*) *Intralobular or perifollicular*

The fibrous tissue of the gland becomes condensed as a result of the epithelial atrophy To this factor is added an increased fibrosis from long continued irritation due to colloid retention or to the cause of the colloid retention.



TEXT FIGURE.—Diagrammatic representation of macroscopic sections through various goitres

(*a*) Normal thyroid Normal secretion

(*b*) Diffuse colloid goitre, with slight perifollicular fibrosis Total secretion of gland normal

(*c*) Diffuse small nodular goitre Diffuse cystic degeneration of a colloid goitre with marked fibrous encapsulation and fibrous atrophy of remaining thyroid tissue Hyposecretion A goitre not unusually found in the congenital goitre of cretins

(*d*) Large localized nodule in a diffuse colloid goitre Little fibroid change except capsular Secretion normal

(*e*) Complete bilateral fibroid atrophy in an old goitre Hyposecretion Myxœdema

(*f*) Complete bilateral atrophic thyroid from a cretin, without goitre Hyper secretion

(*g*) Three adenomatous nodules containing functionally active thyroid tissue resulting from compensatory hypertrophy in a mixed nodular gland, with two colloid nodules Total secretion clinically normal

(*h*) Large primary 'toxic adenoma' in an otherwise normal thyroid Hyposecretion Clinical thyrotoxicosis

(*j*) The diffuse adeno parenchymatous (primary toxic) goitre of Graves' disease

(*k*) Secondary toxic adenoma, developing in a colloid goitre

(a) Perilobular or capsular fibrosis

The surface of the gland on section in the earlier degrees of fibrosis appears more lobular in character due to the perilobular distribution of this early fibrosis (Text-figure, *b*) whilst later each colloid cyst may become surrounded by a dense fibrous tissue capsule so that ultimately, if the colloid cysts are numerous, very little normal thyroid tissue may be left Clinically interlobular sulci appear on the external surface which become more and more appreciable on palpation A diagrammatic section through such a gland is shown in Text figure, *c*, whilst microscopically the great increase in the fibrous tissue capsule is well shown in Plate VII, microphotograph 3 Many of the larger colloid nodules have a white fibrous centre from which fibrous strands radiate outwards towards the periphery

(b) Intralobular or perifollicular fibrosis

The excessive strain on the gland epithelium finally results in its widespread atrophy so that in addition to a perilobular irritant fibrosis a perifollicular and intralobular replacement fibrosis now surrounds the individual degenerating and broken up groups of vesicles (Plate VI, photo 2, and Plate VII, microphotograph 4) The older the tumour the more marked the fibrosis Indeed in some old goitres the gland may be found entirely replaced by fibrous tissue, so that clinically the gland becomes as hard as stone—and clinical signs of myxœdema are likely to appear (Text-figure, *e*, also Plate VI, photo 3) Thus a simple goitre in this stage presents a picture of an increasing degree of fibrosis at first perilobular and later intralobular in character, which is usually accompanied elsewhere in the gland by increasing colloid accumulation in larger and larger cyst formations

IV Calcification and Hæmorrhage

Calcification

In such a degenerate gland calcareous deposits obvious to the touch or to the knife are not infrequently observed One specimen in King George's Medical College Museum [M (*b*) 10 1] shows a large cyst, the wall of which is completely calcified A third common site for calcification is the white fibrous centre of a large colloid nodule

Hæmorrhage

Hæmorrhage may occur into a colloid or solid nodule It may be so small as to only slightly colour part of the gland (*see* Plate VI, photo 1) or so large as to cause a rapid local increase in size.

V Epithelial overgrowth (*a*) *Compensatory nodules, and* (*b*) *Toxic goitres*

(*a*) *Compensatory epithelial nodules*

Small solid adenomata were observed in the diffuse colloid goitres from the U P endemic area and are apparently in the nature of compensatory hypertrophy to provide some thyroxin for an individual on the verge of subthyroidism These mixed goitres are bilateral and nodular on external palpation from old colloid cysts whilst on section they show the small soft opaque white or reddish solid adenomata on a background of the diffuse colloid change (Text-figure, *g*, and Plate VI, photo 2) In a fresh slice section, solid adenomata are granular, moist and raw beef-like in appearance Microscopically, the adenomatous nodule consists of thyroid epithelium in an active secretory phase Large numbers of epithelial cells often in several layers form small round or irregularly-shaped acini with slit-like lumens which are packed together in close contact (Plate VII, microphotograph 5) At periods of greater secretory activity the lumen becomes larger and epithelial papillary processes proliferate rapidly into the watery secretion they contain Into this secretion the epithelial cells often desquamate Such epithelial processes may also proliferate into the colloid vesicles of a colloid goitre (Plate VII, microphotograph 6) The cell nuclei are frequently vesicular, and stain feebly and migrate towards the peripheral basement membrane so that the cells appear columnar in shape In their cytoplasm granules or vacuoles indicating various stages of secretory activity can be made out Collections of lymphocytes aggregate in the lymphoid spaces The connective tissue is increased being cellular and markedly vascular, whence the ' raw beef ' appearance

(*b*) *Epithelial or toxic goitres*

No specimen has been observed of the development in a pre-existing colloid goitre of a large solid adenomata accompanied by clinical thyrotoxicosis (toxic adenoma) (Text-figure, *k*) nor has a toxic adenoma been observed in an otherwise normal gland (Text-figure, *h*) On no occasion has the clinical condition of any individual in an endemic area aroused any suspicion of such a secondary or primary toxic adenoma No example of the diffuse adeno-parenchymatous change of primary exophthalmic goitre (Text-figure, *j*, and Plate VI, photo 4) has been met with in the U P endemic areas In contrast with colloid goitre, the latter type is small, soft, bilateral and not nodular on external palpation, whilst on section such goitres are uniformly homogeneous rawbeef-like and glandular

VI Malignant change

Malignant change arises far more frequently in a diseased than in a normal thyroid, and is especially apt to arise in an old nodular goitre Malignant thyroid

disease is believed to be some ten times more frequent in endemic goitre areas than in goitre-free areas Clinically, the change first starts as a lump which rapidly increases in size and hardness and finally breaks through its capsule Microscopically, one of three main types may be in preponderance (1) adeno carcinoma, (2) papilliferous, and (3) scirrhus The proportion of malignant adenomas to papilliferous adeno-carcinomas is probably somewhere in the neighbourhood of as 9 is to 1 Malignant adenomas probably occur in a still higher proportion in endemic goitre areas whilst in non-goitre areas the proportion is probably definitely lower Scirrhus carcinoma is by far the rarest form In the U P malignant goitres, the cases examined were of the malignant adenoma type [Path Mus Spec No M (*b*) 13]

VII Congenital thyroid hypofunction (*a*) *Colloid goitre, and* (*b*) *Fibroid hypoplasia*

Cretins are frequently born with large colloid goitres, and the mothers of such cretins frequently have colloid goitres of many years standing In the worst cases cretins are born with completely fibroid goitres (Text-figure, *f*) In the second generation colloid goitre in the adult is still generally harmless, but after three, four or five generations the thyroid germplasm has become so weakened that con genital colloid goitre and fibroid hypoplasia which have arisen during intra-uterine life are frequently found in association with infantile myxœdema, deaf mutism, idiocy and cretinism

Summary

1 The endemic goitre of the United Provinces is a diffuse colloid goitre, proceeding to nodular cystic degeneration with extensive fibrosis

2 Such goitres are not usually associated with clinical subthyroidism but when fibrosis is extensive the onset of signs of myxœdema is readily understandable

3 Small adenomatous nodules of thyroid cells in a stage of active secretion may be observed in such goitres and are apparently the result of an effort at compensatory hypertrophy

4 Large adenomas with thyrotoxicosis and adeno-parenchymatous goitres with Graves' disease have not been observed in the U P endemic areas

5 Malignant change is far more common in endemic goitres than in the healthy thyroid, and is usually of an adeno-carcinomatous type

6 Cretins with large colloid or small fibrous goitres are the offspring of maternal subthyroidism acting through several generations

Photo 1

Photo 2

Photo 3

Photo 4

Explanation of Plate VI

Photo 1 —Colloid nodules, with extensive perilobular fibrosis From a male of 30 years who died of cerebral malaria (*a*) Thyroid cartilage (*b*) Cricoid cartilage (*c*) Larynx (*d*) Trachea [K G M C Path Mus Specimen No M (*b*) 11 1]

„ 2 —Colloid goitre Intralobular fibrosis Small nodule of epithelial hyperplasia [K G M C Path Mus Specimen No M (*b*) 10]

„ 3 —Old fibrous goitre From a male of 6 years With some slight calcareous change The right recurrent larvngeal nerve was pressed on with right vocal cord paralysis, dyspnœa and hoarse speech [K G M C Path Mus Specimen No M (*b*) 11]

„ 4 —Thyroid from exophthalmic goitre Dull opaque white in appearance Presented by Sir James Berry from a case in England

Explanation of Plate VII

Microphotograph 1. Normal thyroid and simple hypertrophic goitre

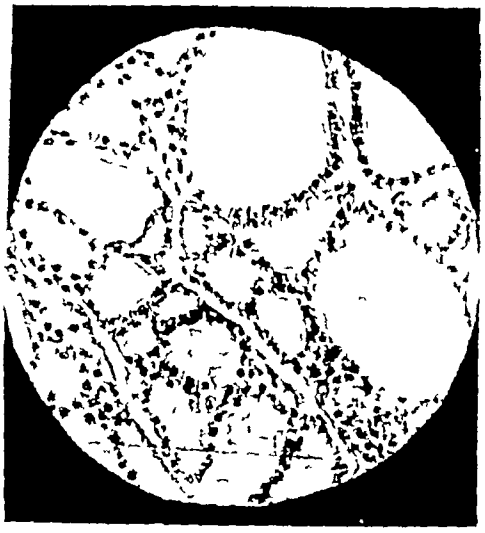
,, 2. *Colloid goitre* Acini vary much in size Colloid abundant Epithelium flattened

,, 3. *Colloid goitre* Perilobular fibrous capsule

,, 4 *Intralobular and perifollicular fibrosis*

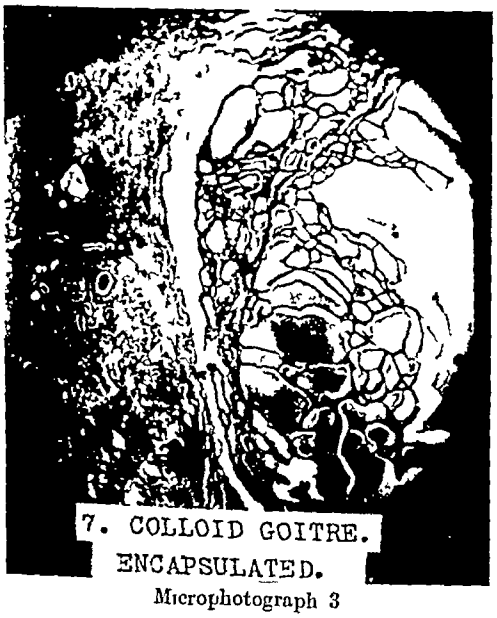
,, 5 Adenomatous nodule, of closely packed epithelial hyperplasia with narrow lumens

,, 6 Epithelial hyperplastic processes projecting into the acini of a colloid goitre

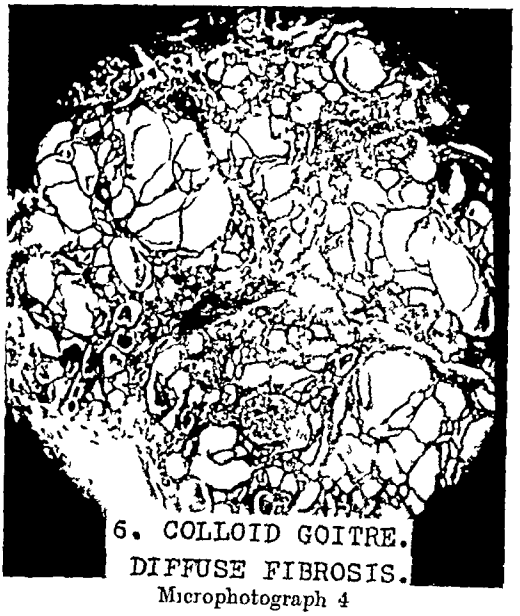


Microphotograph 1

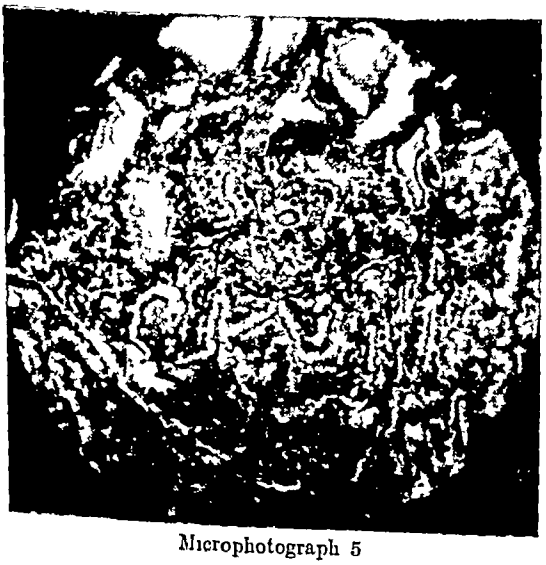
Microphotograph 2



Microphotograph 3



Microphotograph 4



Microphotograph 5

Microphotograph 6

THE DISTRIBUTION AND CAUSE OF ENDEMIC GOITRE IN THE UNITED PROVINCES

Part III.

CAUSE AND SUMMARY OF TREATMENT

BY

LIEUT.-COLONEL H STOTT MD, FRCP, DPH, IMS,
Professor of Pathology, King George's Medical College, Lucknow

[Received for publication, March 7, 1932]

IN the *Indian Journal of Medical Research* for April 1931, Part I of this paper was published, setting forth certain clinical and ætiological observations and showing the connection between the distribution of endemic goitre in the United Provinces and drinking waters of high calcium content

The following is a brief summary of the appropriate sections —

1 In the United Provinces there are four areas of endemic goitre (*a*) The Himalayan area, (*b*) the sub-Himalayan foot-hill area, (*c*) the marshy tract along the north bank of the Gogra river, especially where its Himalayan tributaries join, and (*d*) the Padrauna Tehsil tract of Bhat soil

2 *In all areas*, the evidence for the most intimate connection between goitre population groups and a water-supply of high calcium content has been remarkable This connection has existed for decades of years and is recognized in many instances by the inhabitants themselves

3 *In the Himalayan area*, goitre is endemic in certain scattered villages of the hot damp valleys situated under 5,000 feet above sea-level The goitre rate of affected villages is about 40 per cent, with some 4 per cent of deaf mutes Cretins also are found The water supply is mainly by the mountain springs In the goitre villages examined these springs were traced to limestone rocks, often the so-called 'growing' rocks Vegetation breaks up the soft limestone

and by the carbon dioxide produced in its growth renders insoluble lime soluble as calcium carbonate

4 *The sub-Himalayan foot-hill area* is a centre of minor endemicity The goitre rate is only some 3 to 5 per cent The water supply in the affected villages is from shallow wells and from hill rivulets which course over water-worn limestone boulders and lime kanker from the mountains The villagers most affected are along these rivulets Villages which obtain their supply from the Rapti river are goitre-free The plateau tract to the south of this area is goitre-free except for imported cases

5 *The marshy tract along the north bank of the Gogra river* forms a highly endemic area with areas of hyperendemicity where the Himalayan tributaries join In the worst localities the goitre rate is 70 per cent, whilst numerous cretins and deaf-mutes are met with The area is locally known as the 'abode of fools' and human intelligence is recognized to diminish with length of residence therein Drinking water is mainly from shallow wells in an alluvial soil in which the subsoil water level is very high This soil is inundated in the rains with the water containing much lime from the mountains This lime is deposited with each fresh layer of alluvium The porous soil of this area is similar to that of the Padrauna Tehsil and, like it, contains a very high proportion of calcium, which reaches 9 per cent, or some thirtyfold above the normal

6 *In Padrauna Tehsil area*, the goitre distribution follows almost exactly that of Bhat soil which is a powdery whitish friable alluvium about three feet deep, carried down from the Himalayas by the Gandak river system Bhat soil contains the enormous proportion of 32 per cent of calcium, which is some hundredfold above the normal percentage of calcium in soil The drinking-water from the shallow wells of this soil naturally contain a high calcium content (24 per 100,000) Bhat soil is damp with a high subsoil water-level Into this Bhat soil of the Padrauna Tehsil a tongue of Bangar soil cuts Bangar soil contains only 2 per cent of calcium and the goitre rate on it is approximately only 5 per cent as compared with a 50 per cent rate on Bhat soil Villages drawing their drinking-water from the rapidly flowing great Gandak river escape goitre

7 *The characteristics of a heavily charged lime water* as recognized by some villagers and as connected by them with goitre are its (1) hardness, (2) 'heavy' consistency, (3) 'pricking' (astringent) taste, (4) peculiar 'smell', (5) remaining 'warm' in all seasons whilst good (goitre-free) water keeps cool, (6) white deposit on boiling, (7) becoming 'milky' in the hot weather, (8) turning a brass lota reddish, (9) producing goitre in those who drink it, and (10) if changed for 'good' drinking-water goitres disappear

8 *Certain characteristics of different water supplies* are also associated with goitre-producing or non-goitrous properties in the endemic areas of the United Provinces concerning which the original paper may be consulted

The above abstract serves as an introduction to a few remarks on the possible cause of endemic goitre in the United Provinces which may be considered under the following heads —

(1) An excessive intake of calcium in drinking-water
(2) A deficiency of iodine intake
(3) A bacteriologically impure drinking-water

The cause of endemic goitre in U P

(1) *An excessive intake of calcium in drinking-water*

As has been set out in the original paper, a considerable mass of evidence is available from generations of villagers in the endemic areas of the United Provinces, from local land-holders and educated residents, from authors of the official gazetteers and from the several census reports which incriminate a drinking-water heavily charged with calcium as the cause of goitre in the U P endemic areas Moreover and especially the further evidence collected and checked by the medical personnel of this inquiry working in the actual endemic areas has been to support and extend this belief

Some examples of natural experiments in the endemic areas (e g , ' goitrous ' householders drinking water from a ' goitrous ' well—and of adjoining ' non-goitrous ' householders drinking water from a ' non-goitrous ' well) seem so forceful and are so constantly repeated in different areas that it is difficult not to connect an excess of calcium intake with the cause of endemic goitre in the U P The same view is supported by such further evidence as (1) the remarkable localization of goitre in Padrauna to persons drinking shallow well water drained from Bhat soil which contains 32 per cent of calcium, (2) the goitre-producing Himalayan milky mountain springs and foot-hill mountain streams which in the dry hot weather by evaporation deposit calcium on, and when the growth of vegetation is profuse dissolve calcium from, the soft limestone over which they course, and (3) the limitation of the goitre-producing properties of the Himalayan rivers to a short distance only of their course in the plains after leaving the mountains, that is, until the calcium carried down has been largely deposited with the foot-hill alluvium This whole natural history of the localization of goitre in endemic areas of the U P tends to support the calcium theory of the cause of goitre I have had further personal experience of a similar cause when observing endemic goitre in Kashmir and in Burmah

(2) *Deficiency of iodine intake*

True it may be that the most successful medicinal means for preventing and for reducing endemic goitre is iodine internally, and the iodide of mercury externally True it may be that the normal thyroid contains a large percentage of iodine,

by weight and that in the subthyroidism and cretinism of endemic areas active thyroxin is deficient True it is that diet of the poor villagers of endemic areas is a starvation diet of untold deficiencies besides iodine deficiency But is iodine deficiency therefore the primary cause of endemic goitre? How can the frequently repeated observation of two adjacent villages, one goitrous and one goitre-free, inhabited by equally poverty-stricken ill-nourished villagers consuming the same ill-assorted iodine-deficient starvation diet, be explained when the only detectable difference between them is a 'goitrous' and 'non-goitrous' water supply? The water from both types of these wells were chemically examined by the U P Public Health Department with the uniform report of 'Iodine, nil' All villagers of these U P districts eat crude salt, imported from Bengal and made by evaporating sea water This is their one purchase Rock-salt is dearer and therefore is not utilized Iodine will certainly render the colloid of endemic goitre softer and cause its absorption So will iodine remove gummata but syphilis is not termed a disease of iodine deficiency True the simile is not quite analogous It may be possible that an excess of calcium in the body fixes in some unknown way the available iodine and thus renders the body relatively iodine deficient, but even should this be true in the U P still the primary cause of such a relative deficiency of iodine would in the main appear to be an excess of calcium, and in the endemic areas of the U P this excess of calcium reaches the body cells through the drinking-water

(3) *Intestinal infection from polluted drinking-water*

A large amount of literature and of experimental laboratory work is available to support this theory Certainly in the vast majority of U P endemic areas where drinking-water is obtained from shallow wells such wells are grossly polluted The saturated permeable soil between rivers, the high subsoil water, the proximity of dwelling shelters, the primitive habits of the population, the absence for the most part of any successful attempt to keep the well cement or even brick stained, or properly covered or protected from surface water, the appearance of the water itself, the large amount of potassium permanganate required to pink such wells in times of cholera, the not infrequent epidemics of this disease, the high worm infection rate and much further evidence might be quoted to support the reality of gross pollution But how is one to explain the relative low goitre rate in a village on 'Bangar' soil with a highly polluted shallow well, as compared with the very heavy rate in a village on Bhat (calcium soil) where the shallow wells are at least equally polluted? How is one to explain a heavy goitre rate in hill villages drinking a comparatively bacteriologically pure mountain spring water but milky with calcium? Why are not the vast number of Indian villages remote from the Himalayas drinking water from most grossly polluted shallow wells not affected with goitre? Water pollution may conceivably be a minor contributory ætiological factor but it would

appear in our experience to fall far short of being the primary factor concerned in the production of endemic goitre

The relationship of calcium and of iodine metabolism to the thyroid apparatus

This relationship is not yet clearly understood Calcium metabolism is controlled by the parathyroid whilst the thyroid is the only source of iodine in the body Normally thyroid colloid is of such consistency as to pass readily into the general circulation when required Should the colloid be too rigid it will not pass readily into the circulation but accumulate Lime salts are known to render cell membranes stronger, firmer and less permeable and to render colloid sticky and more concentrated The goitre of the U P endemic areas is a colloid goitre, resulting from colloid concentration It is understandable therefore that with an excessive calcium intake from the limestone drinking-waters of the U P endemic goitre areas, thyroid colloid is made more rigid in consistence, and thus collects in large amounts with the production of a classical colloid goitre whilst later perifollicular fibrosis seals up the colloid within the follicles But whether this result is primarily produced by the action of calcium on the parathyroid or upon the thyroid gland itself or by an ill-understood neutralization of available body iodine it is difficult to hazard an opinion The subcutaneous tissues also thicken and become more rigid with the jelly-like deposit typical of myxœdema The colloid goitre is poor in iodine Oral iodine liquefies the colloid and hence by absorption reduces the size of the goitre and diminishes the myxomatous subcutaneous tissues Per contra, the vesicles of a thyrotoxic goitre either contain some watery colloid or are empty, and such goitres are rich in iodine It is curious that oral iodine appears to cause some colloid retention in such toxic goitres But it would appear true on the whole that the viscosity and therefore the amount of colloid retained varies with the calcium and iodine intake

Further research desirable

So far as the evidence at present available goes, the most likely cause of the endemic goitre of the U P is calcium Nevertheless it is desirable that further research be undertaken to retest the various theories put forward There has been of recent years a mass of experimental evidence produced by laboratory workers It seems that the time has come when further advance would be best gained by testing the practical application of these experiments in the endemic areas of goitre, and to study nature's experiments with man where these are so plentiful A laboratory situated in the centre of an endemic area should produce valuable results

SUMMARY

1 The most probable cause of the endemic colloid goitre of the U P would appear to be an excessive intake of calcium by rendering the thyroid colloid stiffer and the thyroid membranes less permeable Oral iodine renders such rigid colloid more watery and its absorption easier

2 The excessive calcium intake is through drinking-water In certain foothill areas calcium reaches the drinking-water mainly during percolation to shallow wells through alluvial soils heavily impregnated with calcium which has been brought down from soft limestone rocks by mountain rivers and streams Conditions favouring the deposit of such calcium in soil will favour the formation of an area of goitre endemicity In the hills, calcium reaches the drinking-water by solution and suspension from the soft lime rocks over which the spring or stream water flows

3 Calcium is dissolved as calcium carbonate in the water mainly by carbon dioxide set free during the growth or decay of vegetable matter When such a water becomes concentrated as in the hot weather calcium may separate out as a milky suspension When such a water is boiled, carbon dioxide is driven off and lime is deposited as a white chalky powder

4 There is no evidence that has so far become apparent of iodine deficiency nor of intestinal infection from polluted water supplies being the primary cause of goitre in the endemic areas of the United Provinces The diets of almost all villagers in the endemic area are undoubtedly markedly deficient and the waters of almost all wells are undoubtedly markedly polluted but no such clear relationship exists between such deficient diets and such polluted wells and the endemic and non endemic villages as does exist between calcium water supplies and goitre villages

5 It may be, however, that an endemic goitre is due to an upset in the balance between the calcium and the iodine intake In some regions of the world possibly an iodine deficiency may suffice to produce an endemic goitre In other regions a large calcium consumption may be the direct cause In still other regions a deficiency in food iodine may be of itself an insufficient cause unless there exists in addition an excessive calcium intake

SUMMARY OF THE PREVENTION AND TREATMENT OF ENDEMIC GOITRE

A few rules for the prevention and treatment of endemic goitre may be summarized under the following heads —

(*i*) *Residence* —Avoid residence in an endemic area of goitre Such advice, however, is hardly practicable for the population of these areas

(*ii*) *Drinking-water* —Avoid a goitrous drinking-water supply Drinking-water should be taken from non-goitre wells, from the centre of a swiftly flowing river, from rain water when available or theoretically

from distilled water if available If, however, only a hard 'goitrous' water is available, such may be 'softened' or more easily rendered comparatively calcium free by boiling

(*iii*) *Prevention by iodine*—Sodium or potassium iodide in 1½ grain doses may be taken daily for 10 days in the spring and autumn In Switzerland, 0 25 gramme of potassium iodide has been added to each kilogram of salt In Austria, one milligram of iodine per kilogram is mixed by the State in all salt In Gonda, an experiment in the prevention and cure of goitre is at present in progress One minim of the iodine tincture per year of age is given to every child twice weekly as a routine in certain selected schools It is hoped to publish the results subsequently

(*iv*) *Early treatment with iodine*—For the treatment of the early stages of colloid goitre and to prevent their further development, three minims daily of the tincture of iodine or five grains daily of the iodide of potassium may be prescribed for two or three weeks followed by one week's rest, and repeated for two or three months In addition, iodine may be painted externally on the gland, or the red iodide of mercury ointment with an equal amount of lanoline or some cheaper basis rubbed in every other night There is no danger in giving even large doses of iodine to persons with endemic goitre provided they do not start to loose weight or develop a rapid pulse in which case an estimation of the basal metabolism, if desired, provides a decisive test Children stand iodine very well

(*v*) *Treatment of hypothyroidism*—The iodine routine cannot be successful in the old large and in hard fibroid goitres for which, especially if any signs of subthyroidism exist, thyroid extract grain one daily should be prescribed in addition to a course of iodine The weekly weight and pulse rate should be carefully watched In myxœdema with mental deficiency thyroxin must be continued for one to three years and with it mental and physical treatment must also be combined

(*vi*) *Surgical treatment*—Surgery is indicated when pressure symptoms supervene, or when a rapidly enlarging hard nodule appears in an old goitre during the presenile involution period which points to the probability of malignancy

(*vii*) Finally, the essence of the prevention of endemic goitre is the public health measure of the provision of a pure non-goitrous drinking-water supply for the whole population

ANTHROPOMETRIC MEASUREMENTS IN BOMBAY

Part I.

ANTHROPOMETRIC STUDY OF 200 SUBJECTS (100 MALE AND 100 FEMALE)

BY

G M KURULKAR, M B B S

Associate Professor of Anatomy, Seth Gordhandas Sunderdas Medical College Parel, Bombay

AND

V S RAJADHYAKSHA B Sc

Anthropometric Worker under the Indian Research Fund Association

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Introduction

In Europe and America much work is being done in anthropometry References to that subject are found in various standard textbooks of Anatomy and Surgery at present used in India The standard measurements of different body parts mentioned in those books are based on the material obtained from Europe and America alone It is obvious that the physical standards of people in India are different from those standards, and therefore those mentioned in the above books cannot be utilized here for practical purposes An effort therefore is being made to establish those bodily standards for the people permanently staying in India

India is a land of communities A standard of one community may not apply to another community, as these communities are isolated from each other and not allowed to have blood-exchange The modes of life and habits vary in different communities, likewise people living in Northern India cannot be compared with those of the South It is intended, therefore, to study those anatomical variations in different people and to express the differences scientifically It is hoped to study both sexes of each community side by side

Such a study will not only help in establishing the standards, but also will guide in determining whether the people are deteriorating or progressing

The study from its nature is stupendous in a vast country like India To begin, therefore, we started with the observations on the material from amongst the students of our institution The figures worked out from the present study will give averages for a cosmopolitan group of youths of an average age of 22 to 23 years in Bombay drawn mostly from the middle classes

In the present case, the instruments were ordered from P Hermann, Zurich, Switzerland, and the anthropometric work was begun in 1929 As anthropometry does not form a part of the curriculum for University Examinations, the work was conducted for its own merits

Up to June 1931, 200 individuals (100 male and 100 female) were measured The material is mostly from amongst the students of Seth Gordhandas Sunderdas Medical College and the nursing staff of King Edward VII Memorial Hospital of Bombay Thanks are due to these ladies and gentlemen, who so kindly co-operated to make the present task possible by sparing at least one hour for this study I must express my gratitude to Professor R P Koppikar for showing personal interest by giving valuable suggestions from time to time To both Dr Jivraj N Mehta, the Dean of the College, and Professor V R Khanolkar, the acting Dean,

I am indebted for encouragement and active help from time to time to carry on the present work We must acknowledge with thanks that it was owing to Mrs Macarthy, the Matron and Miss Burrows, the Assistant Matron of K E M Hospital, that we could so easily succeed in getting measurements of ladies for inclusion in this present study We also sincerely thank the lady students of our College, Miss Gokhale, Miss Bhide, Miss Nunes, Miss Marathe, Miss Hakim and Miss Pathare, who so enthusiastically helped, even to their great personal inconvenience, in taking measurements of ladies The magnitude of the task of collecting the data and completing and arranging them, so as to make them ready for comparison with such similar material from elsewhere, was not fully realized until after the completion of measurements of the first 100 individuals At the time when the help was most needed the first author was indebted to the Indian Research Fund Association for providing him a sufficient grant for an assistant and sundry expenses

Material and Methods

The male individuals measured in the present study were taken from the 1st M B , B S classes of this College The female individuals represent practically the entire nursing staff of the K E M Hospital, Bombay The geographical regions from which these subjects came are shown as follows —

TABLE I

Native place	Female	Male	Native place	Female	Male
Kathiawar		10	South India	1	
Gujrath	2	12	Madras	1	1
Deccan	54	38	Bengal	1	3
Goa	5	11	Assam	1	
Karwar		3	U P	4	5
Kanara	3	1	Punjab		1
Karnatak		2	Rajputana		3
Mysore	1		C P	7	9
Coimbatore	1		Rangoon	1	
Talichery	1		Unrecorded	5	1
Travancore	2				
Mangalore	10				
				100	100

In the case of male subjects the mean age of the group was 22 91 years with a range from 18 to 31 years The stock of the subjects according to the caste was predominantly Hindu (Indians), that of Christian (Indian) rank stood second

In the case of female subjects the mean age of the group was 23 69 years with a range from 18 to 43 years

The stock of the subjects according to the caste was predominantly Hindu (Indians), that of Anglo-Indian rank stood second and of Christian (Indians) rank stood third The actual distributions are tabulated below —

TABLE II

Nationality	Female	Male	Nationality	Female	Male
Deshastha Brahmin	2	16	Naidu		1
Konkanasth ,,	8	7	Rajput		1
Saraswat ,,	9	9	Bengali		3
Gujrathi ,,		6	Lohana		1
Hindi ,,		5	Gupta		1
Kanauj ,,	1		Native Christian	27	14
U P Kshatriya	1		Mohammedan Bhora		1
Maratha	3	10	,, Sunni		2
Daivadnya	1	1	Parsees	7	
C K Prabhu	3	2	Jews	4	2
Pathare Prabhu		1	Anglo Indians	29	
Khatri		1	English	1	
Khassi	1		Portuguese	1	
Bania		9	European Jew	1	
Jain		6			
Telgu	1				
Brahmo Kshatriya		1		100	100

The measurements were made with the clothes off, wherever necessary, in the case of male subjects, while in the case of female subjects they were made over everyday clothing, the shoes alone being removed The subject upon arrival was told the purpose of the study and asked to remove the shoes and stand in a military attitude The procedure was the same for each one of the two hundred individuals and followed a form booklet of 17 pages The first page of this furnished space for the name, native place, birth date, nationality and general description of the skin, hair and iris of the subject The rest of the pages held the principal physical measurements listed in Table III(*a*) Drawings of the right hand and foot were placed on separate sheets of paper with the respective serial number of the subjects under measurement.

TABLE III(*a*)

Measurements and observations

		Page			Page
Caste		158	22	Frontal height, physiognomic	188
Native place		157	23	Height of mucous lips	189
Age		169	24	Height of entire upper lip	189
Skin, colour of		258	25	Height of entire lower lip	190
Hair, colour of		259	26	Height of chin	190
Eyes, colour of		258	27	Physiognomic ear length	191
1	Maximum head length	170	28	Physiognomic ear breadth	191
2	Glabella inion length	171	29	Morphological ear length	192
3	Maximum head breadth	171	30	Morphological ear breadth	193
4	Least frontal breadth	172	31	Horizontal circumference of head	194
5	Bizygomatic breadth	173	32	Sagittal arc	194
6	Bigonial breadth	175	33	Transverse arc	195
7	Biauricular breadth	176	34	Height, Vertex (total stature)	202
8	Bimastoid breadth	176	35	Height, Tragus	206
9	Biocular breadth	177	36	Height, Gnathion	207
10	Interocular breadth	177	37	Height, Suprasternal	208
11	Nasal breadth	178	*38	Height, Thelion	208
12	Oral breadth	179	*39	Height, Mesosternal	209
13	Auricular height	179	*40	Height, Omphalion	209
14	Height Vertex to sub nasal	182	*41	Height, Symphysion	210
15	Physiognomic facial length	183	42	Height, Ilio cristal	210
16	Morphological facial length	184	43	Height, Ilio spinal	211
17	Physiognomic superior facial length	185	44	Height, Vertebral	211
18	Morphological superior facial length	185	45	Height, Lumbale	212
			46	Height, Acromian	212
19	Nasal length in ground plan	186	47	Height, Radial	230
20	Nasal length profile	187	48	Height, Stylion	232
21	Nasal height from face	188	49	Height, Dactylion	235

TABLE III(*a*)—*concld*

		Page
50	Height Trochanterion	210
50	Height Tibiale	211
52	Height Sphenion	211
53	Sitting height, Vertex	213
54	Sitting height, Tragus	214
55	Sitting height, Suprasternal	215
56	Sitting height, Vertebral	215
57	Sitting height, Ilio cristal	216
58	Arm stretch	229
59	Biacromial diameter	216
60	Breadth of shoulders	219
*61	Bimammillary diameter	220
62	Ilio cristal diameter	217
63	Ilio spinal diameter	221
Telgu	hanteric diameter	218
Brahmo Kshav	l diameter of	222
	I	
The measurement	of thorax	222
of male subjects, w		
day clothing, the shoes	rax	223
purpose of the study an		
The procedure was the s		223
form booklet of 17 pages		
place, birth date, nationa		
subject The rest of t		
in Table III(*a*) Draw		
s of paper with the r		

		Page
71	Bicondylar diameter at knee	252
72	Bimalleolar diameter at ankle	253
73	Girth of neck across larynx	224
*74	Girth of thorax plane I	203
*75	Girth of thorax plane II	204
*76	Girth at waist least	206
77	Girth of upper arm, maximum	231
78	Girth across contracted biceps	232
79	Girth of forearm, greatest	233
80	Girth of wrist, least	237
*81	Girth of thigh, maximum	242
82	Girth of thigh, middle	243
83	Girth of thigh, least	244
84	Girth of calf	246
85	Girth of ankle, least	248
86	Length of hand	238
87	Breadth of hand	239
88	Length of foot	249
89	Breadth of foot	250
90	Weight of body	203
91	Counter tracing of palm	†
92	Counter tracing of foot	

included in the present paper

TABLE III(*b*)

List of indices computed from above data

		Page
1	Maximum head breadth / Maximum head length	172
2	Auricular height / Maximum head length	180
3	Glabella inion length / Sagittal arc	195
4	Biauricular breadth / Transverse arc	196
5	Least frontal breadth / Maximum head breadth	173
6	Morphological facial length / Bizygomatic breadth	184
7	Morphological superior facial length / Bizygomatic breadth	186
8	Least frontal breadth / Bizygomatic breadth	174
9	Bigonial breadth / Bizygomatic breadth	175
10	Nasal breadth / Nasal length in ground plan	187
11	Bizygomatic breadth / Maximum head breadth	174
12	Auricular height / Mean of maximum head length *plus* breadth	180
13	Auricular height / Maximum head breadth	181
14	(Maximum head length *plus* Maximum head breadth *plus* auricular height) / 3	181
15	Bizygomatic breadth / Physiognomic facial length	183
16	Physiognomic ear breadth / Physiognomic ear length	192
17	Morphological ear breadth / Morphological ear length	193

TABLE III(*b*)—*contd*

		Page
18	Mean of biocular breadth and interocular breadth / Maximum head breadth	178
19	Arm stretch / Total stature	230
20	Acromian *minus* stylion / Total stature	235
21	Upper arm / Total arm with hand	236
22	Lower arm / Total arm with hand	236
23	Lower arm / Upper arm	233
24	Hand breadth / Hand length	240
25	Foot breadth / Foot length	251
26	Sitting height / Total stature	213
27	Thorax plane II, dorso ventral diameter / Thorax plane II, tranverse diameter	224
28	Ilio cristal diameter / Breadth of shoulders	219
29	Hand length / Forearm	239
30	Lower leg / Thigh	245
31	Foot length / Lower leg	250
32	Length of entire arm with hand / Entire leg	249
33	Upper arm *plus* forearm / Length of thigh *plus* length of lower leg	245
34	Upper arm / Length of thigh	241
35	Forearm / Length of lower leg	246

TABLE III(*b*)—*concld*

		Page
36	Girth of upper arm / Length of upper arm	231
37	Girth of forearm / Length of forearm	234
38	Girth of forearm / Girth of upper arm	234
39	Girth of wrist / Girth of upper arm	237
40	Girth of wrist / Girth of forearm	238
*41	Girth of thigh, greatest / Length of thigh	242
42	Girth of calf / Length of lower leg	247
*43	Girth of calf / Maximum girth of thigh	247
44	Girth of ankle / Girth of calf	248
*45	Bimammillary diameter / Biacromial diameter	220
46	Ilio spinal diameter / Ilio cristal diameter	221
47	Ilio cristal diameter / Biacromial diameter	217
48	Bitrochanteric diameter / Biacromial diameter	218
49	Leg length / Trunk length	214
*50	Total stature *minus* (maximum girth of thorax in centimetres *plus* weight in kilograms)	205
51	Weight in grammes / Stature in centimetres	203
52	$\frac{\sqrt[3]{\text{Weight in grammes}}}{\text{Total height in centimetres}}$	204

In taking the girths of the thigh of the females the authors were assisted by the lady students of this College In the case of female subjects, measurements and indices marked with an asterisk (*) had to be omitted

In the calculation of means, standard deviations, coefficients of variations and indices and other computations, use was made of a slide-rule and logarithmic tables An ample use was made for reference of Pearl's 'Medical Biometry and Statistics'

A description of the instruments used and of the methods applied in taking the measurements is given below —

Instruments.

(1) Anthropometer —A rod of 200 cm in length detachable in 4 parts and having a fixed horizontal rod at the top and another adjustable, sliding horizontal bar Any lengths and breadths of trunk can be measured by this instrument

(2) A slide compass for measuring smaller dimensions of face, eye, hand and foot

(3) A calipers —To measure head dimensions

(4) A pelvimeter for pelvic and thorax antero-posterior diameters

(5) Osteometric board for measuring the length of the foot

(6) A balance registering weights in kilogrammes

Method of taking Measurements and Observations.

(1) Maximum cranial length, a distance between glabella and opisthocranion taken by calipers, by keeping one tip of calipers on glabella and the other allowed to travel over the median line of the head, the widest distance being registered (Table V)

(2) Glabella-inion length (Table VI)

(3) Maximum head breadth, taken in a horizontal plane allowing the two tips of the calipers diverge over the sides of the head Widest divergence recorded (Table VII)

(4) Least frontal breadth, distance between points on frontotemporal lines where they are nearest to each other (Table IX)

(5) Bizygomatic breadth, the distance between the farthest points on zygomatic arches of both the sides (Table XI)

(6) Bigonial breadth, the distance between the two angles of the mandible (Table XIV)

(7) Biauricular breadth, the distance between the notches just above the tragus of the ears of both sides (Table XVI)

(8) Bimastoid breadth, the distance between the farthest points on mastoid processes of both sides (Table XVII)

(9) Biocular breadth, the distance between the external canthi of both the eyes (Table XVIII)

(10) Interocular breadth, the distance between the internal canthi of both the eyes (Table XIX)

(11) Nasal breadth, the distance between the farthest points on the ala of the nose (Table XXI)

(12) Oral breadth, the distance between the corners of the mouth, in a quiet condition (Table XXII)

(13) Auricular height, taken by taking two measurements, one, the height of the vertex from ground (projection), two, the height of the notch above tragus of the ear from ground (projection), and subtracting 2 from 1 The notch above tragus corresponds to the base of the skull (Table XXIII)

(14) Height, vertex to the sub-nasal, this height is similarly taken as 13 Height from ground to the point of the angle between the septum of the nose and the surface of the upper lip, is subtracted from the height of the vertex (Table XXVIII)

(15) Physiognomic facial length, the distance from the median point in the line of the hair, to the gnathion, the median point on the chin (Table XXIV)

(16) Morphological facial length, the distance between the nasion and gnathion (Table XXXI)

(17) Physiognomic superior facial length, the distance between the nasion and the median point of the oral slit (Table XXXIII)

(18) Morphological superior facial length, the distance between the nasion and the median point on the upper alveolar border (Table XXXIV)

(19) Nasal length in ground plan, the distance from nasion to the lowest point on the pyriform aperture of the nose (Table XXXVI)

(20) Nasal length along profile, the distance from nasion to the point of the nose (Table XXXVIII)

(21) Nasal height, projection from face, the distance from the point sub-nasal to the point of the nose (Table XXXIX)

(22) Frontal height, physiognomic, the distance from nasion to the median point on the line of the hair (Table XL)

(23) Height of mucous lips, the distance from the median point on the lower border of the mucous surface of the lower lip to the median point of a line drawn across the boundary of the mucous surface of the upper lip, tangent to the curves (Table XLI)

(24) Height of the entire upper lip, the distance from the sub-nasal to the median point of the oral slit (Table XLII)

(25) Height of the entire lower lip, the distance from the median point of the oral slit to the depression below the lower lip in the median line (Table XLIII)

(26) Height of the chin, the distance of the median point of the oral slit to the gnathion (Table XLIV)

(27) Physiognomic ear length, the distance between the highest point on the helix and the lowest point on the lobule (Table XLV)

(28) Physiognomic ear breadth, the distance between two lines parallel to the long axis of the ear, one of these lines being tangent to the anterior, the other to the posterior border of the helix (Table XLVI)

(29) Morphological ear length, the distance between the notch above the tragus and Darwin's tubercle on the edge of the helix (Table XLVIII)

(30) Morphological ear breadth, the distance between the point where the ear attaches to the head above and the point where the ear attaches to the side of the head, below (Table XLIX)

(31) Horizontal circumference of the head, measured by putting one end of the tape over glabella and carrying it along the side of the head horizontally over the farthest point on the back of the head from glabella, and bringing it back to the glabella (Table LI)

(32) Sagittal arc, measured by putting the tape from glabella over the top of the head to the inion (Table LII)

(33) Transverse arc, measured by putting the tape from the notch above the tragus of the ear on one side to that of the other side over vertex (Table LIV)

(34) Height, Vertex, total stature projection from ground (Table LVI)

(35) Height, Tragus, projection from ground to the notch just above tragus (Table LXIV)

(36) Height, Gnathion, projection from ground (Table LXV)

(37) Height, Suprasternal, projection from ground to the suprasternal notch (Table LXVI)

(38) Height, Thelion, from ground to the mid-point of the nipple (Table LXVII)

(39) Height, Mesosternal, the distance from the ground to the median point, on the sternum, of the line which connects the sternocostal articulation of the two 4th ribs (Table LXVIII)

(40) Height, Omphalion, the distance from the ground to the umbilicus (Table LXIX)

(41) Height, Symphysion, the distance from the ground to the middle point in the upper border of the pubic arch, at the symphysis (Table LXX)

(42) Height, Ilio-cristal, the distance from the ground to the most lateral point on the iliac-crest (Table LXXI)

(43) Height, Ilio-spinal, the distance from the ground to the anterior superior spine of the ilium (Table LXXII)

(44) Height, Vertebral, taken from the ground to the spinoses process of the 2nd cervical vertebra (Table LXXIII)

(45) Height, Lumbale, the distance from the ground to the 5th lumbar spine (Table LXXIV)

(46) Height, Acromial, the distance from the ground to the most lateral point of the acromial process, felt through the skin (Table LXXV)

(47) Height, Radial, the distance from the ground to the plane of the top of the head of the radius (Table CII)

(48) Height, Stylion, the distance from the ground to the distal margin of the styloid process of the radius (Table CVI)

(49) Height, Dactylion, the distance from the ground to the point of the middle finger of the hanging arm (Table CXI)

(50) Height, Trochanterion, the distance from the ground to the highest point of the trochanter major as felt through the skin (Table CXXII)

(51) Height, Tibiale, the distance from the ground to the medial separation between femur and tibia, at the medial condylar margin of the latter (Table CXXIII)

(52) Height, Spherion, height of the tip of the medial malleolus from the ground (Table CXXIX)

(53) Sitting height, Vertex, the person is made to sit straight on a flat stool, with his feet resting on a low stool, so that his ischial tuberosities come in contact with the surface of the stool Height of the vertex from the level of the upper surface of the stool measured (Table LXXVI)

(54) Sitting height, Tragus, taken in the same manner as above (Table LXXIX)

(55) Sitting height, Suprasternale (Table LXXX)

(56) Sitting height, Vertebral, taken in the above manner, from the plane of the stool to the Spinous process of 2nd cervical vertebra (Table LXXXI)

(57) Sitting height, Ilio-cristale (Table LXXXII)

(58) Arm stretch taken by an anthropometer placed behind against the shoulders of the person, who has to stretch his arms by pushing the two arms of the instrument by the tips of his middle fingers The maximum distance is the arm stretch (Table C)

(59) Biacromial diameter, the distance between the external tips of the acromial ends of the spines of both the scapulæ (Table LXXXIII)

(60) Breadth of shoulders between deltoids (Table LXXXVIII)

(61) Bimammillary diameter, the distance between two nipples (Table XC)

(62) Ilio-cristal diameter, the distance between the most external points on the crests of the ilia (Table LXXXIV)

(63) Ilio-spinal diameter, the distance between two anterior superior iliac spines (Table XCII)

(64) Bitrochanteric diameter, the distance between the lateral surfaces of greater trochanters of femur, when a person stands in the military fashion (Table LXXXVI)

(65) Dorso-ventral diameter of thorax plane I (at the level of the Xiphoid process), a distance between the point on the anterior median line as the Xiphoid process in a horizontal plane when a person is standing and another on the back in the posterior median line in the same plane (Table XCIV)

(66) Transverse diameter of thorax plane I Two arms of the anthropometer resting on the sides of the chest on a horizontal plane at the level of Xiphoid process (Table XCV)

(67) Dorso-ventral diameter of thorax plane II in the same way as 65, but at the level Mesosternale (Table XCVI)

(68) Transverse diameter of thorax plane II in the same way as 66 but in the level of Mesosternale (Table XCVII)

(69) Bicondylar diameter at elbow, between the epicondyles of the lower end of the humerus (Table CXLIII)

(70) Bistyloid diameter of wrist, between the most external points on the lower ends of radius and ulna (Table CXLIV)

(71) Bicondylar diameter at knee a distance between the two epicondylar eminences at the lower end of femur (Table CXLV)

(72) Bimalleolar diameter at ankle, a distance between the most lateral points on the lower ends of tibia and fibula of one side (Table CXLVI)

(73) Girth of neck by tape across larynx (Table XCIX)

(74) Girth of thorax plane I at the level of Xiphoid process (Table LXII)

(75) Girth of thorax plane II at the level of Mesosternale (Table LX)

(76) Girth of waist (least girth of body) (Table LXIII)

(77) Girth of upper arm (greatest when relaxed) (Table CIII)

(78) Girth across contracted biceps (Table CV)

(79) Girth of forearm, greatest when relaxed (Table CVIII)

(80) Girth of wrist, least (Table CXV)

(81) Girth of thigh, greatest in or about the gluteal fold (Table CXXV)

(82) Girth of thigh, middle (Table CXXVII)

(83) Girth of thigh, least (just above knee) (Table CXXVIII)

(84) Girth of calf (Table CXXXIII)

(85) Girth of ankle, least (Table CXXXVI)

(86) Length of the hand taken by a slide-compass between the lower end of radius and the tip of the middle finger (Table CXVIII)

(87) Breadth of hand, at the heads of the metacarpal bones (Table CXX)

(88) Length of the foot taken in an osteometric board by keeping right foot in contact with the front board and medial side touching the sides and by applying wooden piece to heel and recording the measurements from the graph-paper (Table CXXXIX)

(89) Breadth of foot between the heads of the metatarsal bones (Table CXLI)

(90) Weight of the body on a balance recording kilograms In case of males with scanty clothing In females with full clothing on (Table LVII)

(91) Counter tracing of the hand by keeping the hand quite flat with the palm on the paper and tracing by a pencil, whose one half wooden cover is split up so that the lead comes in contact with the outline of the hand

(92) Counter tracing of the foot taken as above

All appendicular measurements are taken on the right side

These measurements will be discussed in groups First the head measurements, then the trunk measurements and lastly the measurements of the appendages

Abbreviations used in the Tables

(Each table indicates the distribution of individuals in Class-groups)

Z = Frequency of individuals
P E = Probable error
S D = Standard deviation
C V = Coefficient of variation
mms = Millimetres
X = Percentage of the male mean to the female mean

Ages

TABLE IV

Ages Years	Z Female	Z Male	Ages Years	Z Female	Z Male	Ages Years	Z Female	Z Male
18—19	3	2	27	5	3	36	0	
19	10	3	28	6	0	37	0	
20	16	12	29	2	0	38	0	
21	19	21	30	2	0	39	0	
22	11	20	31	4	1	40	0	
23	5	15	32	0		41	0	
24	5	11	33	0		42	0	
25	4	6	34	0		43	1	
26	6	6	35	1				
							100	100
Mean and P E			Female 23 7 ± 0 27			Male 22 9 ± 0 15		

Study of Head.

(a) Tables of measurements and indices of head

Table V

Maximum head length

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
160—161 9	2		176	17	1	192		7
162	1		178	12	6	194		7
164	3		180	13	10	196		9
166	2		182	6	7	198		2
168	8		184	6	9	200		2
170	7		186	1	9	202		1
172	10		188	4	20	204		1
174	6	3	190	2	6			
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	176 4 ± 0 43	187 9 ± 0 45	−11 5 ± 0 62	
S D and P E (mms)	6 5 ± 0 30	6 6 ± 0 32	− 0 1 ± 0 44	93 8
C V and P E (percent)	3 7 ± 0 18	3 5 ± 0 17	+ 0 2 ± 0 24	

TABLE VI

Head length (Glabella-inion length)

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
156—157 9	2		170	14	0	184	1	14
158	3		172	9	2	186	1	10
160	2		174	13	6	188	2	5
162	3		176	10	4	190		7
164	6		178	13	10	192		5
166	5	1	180	2	15	194		6
168	8	0	182	6	14	196		1
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	172 6 ± 0 46	183 6 ± 0 39	− 11 0 ± 0 60	94 3
S D and P E (mms)	6 8 ± 0 33	5 9 ± 0 28	+ 0 9 ± 0 43	
C V and P E (per cent)	3 9 ± 0 19	3 2 ± 0 16	+ 0 8 ± 0 25	

TABLE VII

Head width

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
123—124 9	1		137	10	3	151	5	7
125	0		139	17	8	153	0	5
127	0		141	13	6	155	1	6
129	1		143	7	10	157		4
131	4	1	145	7	25	159		1
133	9	2	147	6	13			
135	13	2	149	6	7			
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	140 5 ± 0 40	146 3 ± 0 39	− 5 9 ± 0 55	95 8
S D and P E (mms)	5 9 ± 0 28	5 7 ± 0 27	+ 0 1 ± 0 39	
C V and P E (per cent)	4 1 ± 0 2	3 9 ± 0 19	+ 0 2 ± 0 27	

Table VIII

Cephalic index $= \frac{\text{Head width} \times 100}{\text{Head length}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
65 5—67 4		1	75 5	16	17	85 5	5	3
67 5		3	77 5	15	17	87 5	6	
69 5		3	79 5	11	11	89 5	1	
71 5	8	13	81 5	14	13	91 5	2	
73 5	12	8	83 5	10	8			
							100	100

	Female	Male	Difference	λ
Mean and P E	80 ± 0 31	77 9 ± 0 30	+ 2 1 ± 0 44	
S D and P E	4 7 ± 0 22	4 5 ± 0 21	+ 0 2 ± 0 31	102 7
C V and P E	5 7 ± 0 27	5 7 ± 0 27	0 0 ± 0 39	

Table IX

Frontal breadth

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
89—90 9	2	1	99	24	7	109	1	8
91	1	2	101	25	15	111		2
93	3	2	103	16	18	113		1
95	8	9	105	5	16		100	100
97	11	9	107	4	10			

	Female	Male	Difference	X
Mean and P E (mms)	100 4 ± 0 25	102 5 ± 0 33	− 2 2 ± 0 42	
S D and P E (mms)	3 6 ± 0 17	5 0 ± 0 24	− 1 3 ± 0 29	97 8
C V and P E (per cent)	3 6 ± 0 17	4 8 ± 0 23	− 1 2 ± 0 29	

TABLE X

Transverse fronto-parietal index = $\frac{\text{Least frontal breadth} \times 100}{\text{Maximum head breadth}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
59 5—61 4		2	67 5	13	12	75 5	6	5
61 5		3	69 5	27	21	77 5	1	2
63 5	2	9	71 5	26	15	79 5		2
65 5	7	15	73 5	18	14		100	100

	Female	Male	Difference	X
Mean and P E	71 5 ± 0 19	70 2 ± 0 28	+ 1 3 ± 0 35	
S D and P E	2 8 ± 0 13	4 2 ± 0 20	− 1 4 ± 0 24	101 8
C V and P E	3 9 ± 0 19	6 0 ± 0 29	− 2 1 ± 0 34	

TABLE XI

Bizygomatic breadth

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
112—113 9	1		124	17	6	136	0	7
114	0		126	20	11	138	1	7
116	2		128	8	15	140		1
118	3		130	12	13	142		1
120	17	1	132	8	15	144		1
122	6	1	134	5	21		100	100

	Female	Male	Difference	X
Mean and P E (mms)	126 0 ± 0 32	131 7 ± 0 35	− 5 7 ± 0 48	
S D and P E (mms)	4 8 ± 0 23	5 2 ± 0 25	− 0 5 ± 0 34	95 6
C V and P E (per cent)	3 8 ± 0 18	4 0 ± 0 19	− 0 2 ± 0 26	

Table XII

Zygomatic frontal index = $\frac{\text{Least frontal breadth} \times 100}{\text{Bizygomatic breadth}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
65 5—67 4		3	73 5	1	10	81 5	21	10
67 5		2	75 5	12	21	83 5	4	2
69 5	1	6	77 5	28	22	85 5	2	1
71 5	1	1	79 5	27	19		100	100

	Female	Male	Difference	λ
Mean and P E	79 7 ± 0 23	77 4 ± 0 27	+ 2 4 ± 0 36	103
S D and P E	3 4 ± 0 16	4 1 ± 0 19	− 0 7 ± 0 25	
C V and P E	4 2 ± 0 20	5 2 ± 0 25	− 1 0 ± 0 32	

Table XIII

Transverse craniofacial index = $\frac{\text{Bizygomatic breadth} \times 100}{\text{Maximum head breadth}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
62 5—64 4		1	76 5		0	90 5	23	18
64 5		2	78 5		1	92 5	11	11
66 5		4	80 5	2	1	94 5	5	4
68 5		5	82 5	3	0	96 5	0	2
70 5		6	84 5	13	9	98 5	2	
72 5		4	86 5	14	11		100	100
74 5		2	88 5	27	19			

	Female	Male	Difference	X
Mean and P E	89 8 ± 0 22	85 3 ± 0 19	+ 4 4 ± 0 30	104 6
S D and P E	3 3 ± 0 16	2 9 ± 0 14	+ 0 4 ± 0 21	
C V and P E	3 7 ± 0 18	3 4 ± 0 16	+ 0 3 ± 0 24	

TABLE XIV

Bigonial breadth

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
70—71 9	2		84	11	11	98		2
72	2	1	86	11	12	100		3
74	5	0	88	7	11	102		2
76	6	2	90	4	18	104		1
78	7	3	92	3	6	106		1
80	19	6	94		6			
82	20	7	96		8		100	100

	Female	Male	Difference	X
Mean and P E (mms)	82 4 ± 0 32	89 0 ± 0 44	− 6 6 ± 0 54	
S D and P E (mms)	4 7 ± 0 23	6 4 ± 0 31	− 1 7 ± 0 38	92 7
C V and P E (per cent)	5 7 ± 0 27	7 2 ± 0 35	− 1 5 ± 0 44	

TABLE XV

Zygomatico-mandibular index $= \frac{\text{Bigonial breadth} \times 100}{\text{Bizygomatic breadth}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
49 5—51 4	1		63 5	22	12	77 5		0
51 5	1		65 5	30	17	79 5		1
53 5	0		67 5	12	15	81 5		1
55 5	1	2	69 5	6	14	83 5		1
57 5	4	2	71 5	7	9			
59 5	4	7	73 5	2	5			
61 5	10	9	75 5		5		100	100

	Female	Male	Difference	X
Mean and P E	65 8 ± 0 28	67 9 ± 0 35	− 2 1 ± 0 45	
S D and P E	4 1 ± 0 20	5 2 ± 0 25	− 0 7 ± 0 32	91 8
C V and P E	6 2 ± 0 30	7 7 ± 0 37	− 1 0 ± 0 48	

Table XVI

Auricular breadth

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
109—110 9	2		123	11	12	137	1	4
111	2		125	11	16	139		2
113	5		127	10	13	141		0
115	9	1	129	3	22	143		1
117	2	0	131	4	10			
119	15	6	133	2	6		100	100
121	16	2	135	1	5			

	Female	Male	Difference	X
Mean and P E (mms)	122 4 ± 0 38	128 4 ± 0 32	− 6 0 ± 0 50	
S D and P E (mms)	5 6 ± 0 27	4 8 ± 0 23	− 0 7 ± 0 35	95 3
C V and P E (per cent)	4 5 ± 0 22	3 7 ± 0 18	− 0 8 ± 0 28	

Table XVII

Bimastoid breadth

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
109—110 9	2		123	10	7	137	1	3
111	0		125	11	14	139		1
113	5		127	9	16	141		0
115	5	1	129	8	10	143		1
117	9	1	131	2	13			
119	21	6	133	1	12		100	100
121	15	7	135	1	8			

	Female	Male	Difference	X
Mean and P E (mms)	121 9 ± 0 36	128 5 ± 0 36	− 6 6 ± 0 45	
S D and P E (mms)	5 3 ± 0 25	5 3 ± 0 25	0 0 ± 0 36	94 6
C V and P E (per cent)	4 3 ± 0 21	4 1 ± 0 20	+ 0 2 ± 0 67	

TABLE XVIII

Biocular breadth

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
78—79 9		1	88	13	9	98	2	7
80	1	0	90	24	21	100		1
82	1	0	92	16	22	102		1
84	11	2	94	13	17			
86	11	6	96	8	13		100	100

	Female	Male	Difference	X
Mean and P E (mms)	90 4 ± 0 26	92 5 ± 0 25	− 2 1 ± 0 36	
S D and P E (mms)	3 8 ± 0 18	3 8 ± 0 18	0 0 ± 0 26	97 5
C V and P E (per cent)	4 2 ± 0 20	4 1 ± 0 20	+ 0 1 ± 0 28	

TABLE XIX

Interocular breadth

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
23—24 9	2	1	29	32	21	35	3	2
25	5	3	31	28	29	37	1	1
27	15	17	33	14	26			
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	29 5 ± 0 21	30 8 ± 0 17	− 1 3 ± 0 27	
S D and P E (mms)	3 2 ± 0 15	2 5 ± 0 12	+ 0 7 ± 0 19	95 7
C V and P E (per cent)	10 8 ± 0 52	8 0 ± 0 39	+ 2 7 ± 0 65	

TABLE XX

Interpupillo-cranial index = $\frac{\text{Mean of biocular breadth and interocular breadth} \times 100}{\text{Maximum head breadth}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
35 5—37 4	2		41 5	25	31	47 5	1	1
37 5	8	15	43 5	31	20			
39 5	16	22	45 5	14	8		100	100

	Female	Male	Difference	X
Mean and P E	43 0 ± 0 17	42 2 ± 0 16	+ 0 8 ± 0 23	
S D and P E	2 8 ± 0 12	2 3 ± 0 11	+ 0 5 ± 0 16	102
C V and P E	5 7 ± 0 27	5 5 ± 0 26	+ 0 2 ± 0 38	

TABLE XXI

Nasal breadth

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
27—28 9	4		33	27	17	39		11
29	20	2	35	17	36	41		2
31	24	10	37	8	22		100	100

	Female	Male	Difference	X
Mean and P E (mms) .	32 6 ± 0 17	35 6 ± 0 16	− 3 0 ± 0 24	
S D and P E (mms) .	2 5 ± 0 12	2 4 ± 0 11	+ 0 2 ± 0 17	91 5
C V and P E (per cent) .	7 8 ± 0 37	6 6 ± 0 32	+ 1 2 ± 0 49	

TABLE XXII

Oral breadth

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
29—30 9		1	39	4	0	49	13	25
31		0	41	17	2	51	7	13
33		0	43	16	11	53	1	8
35		0	45	21	13	55	2	2
37	2	0	47	17	24	57		1
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	45 7 ± 0 25	48 3 ± 0 24	− 2 6 ± 0 34	
S D and P E (mms)	3 7 ± 0 18	3 5 ± 0 17	+ 0 2 ± 0 26	94 5
C V and P E (per cent)	8 1 ± 0 39	7 2 ± 0 35	+ 0 9 ± 0 52	

TABLE XXIII

Auricular height

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
101—102 9	1		119	13	4	137		1
103	0		121	10	8	139		3
105	1		123	14	10	141		0
107	0		125	14	13	143		0
109	5	1	127	6	13	145		1
111	2	0	129	7	16	147		0
113	2	1	131	4	6	149		1
115	9	5	133	2	8			
117	8	4	135	2	5		100	100

	Female	Male	Difference	X
Mean and P E (mms)	125 7 ± 0 42	127 0 ± 0 45	− 1 3 ± 0 61	
S D and P E (mms)	6 2 ± 0 29	6 7 ± 0 32	− 0 5 ± 0 43	98 8
C V and P E (per cent)	4 9 ± 0 23	5 2 ± 0 25	− 0 4 ± 0 34	

Table XXIV

Length auricular height index $= \frac{\text{Auricular height} \times 100}{\text{Maximum head length}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
55 5—57 4		1	65 5	11	21	75 5	7	1
57 5	1	2	67 5	16	20	77 5	0	
59 5	3	2	69 5	23	18	79 5	1	
61 5	6	6	71 5	13	6			
63 5	10	18	73 5	6	5		100	100

	Female	Male	Difference	X
Mean and P E	69 1 ± 0 25	67 5 ± 0 25	+ 1 6 ± 0 35	
S D and P E	3 7 ± 0 18	3 7 ± 0 18	0 0 ± 0 25	102 2
C V and P E	5 3 ± 0 26	5 5 ± 0 26	− 0 1 ± 0 36	

Table XXV

Mean height index $= \frac{\text{Auricular height} \times 100}{\text{Mean of maximum head length and breadth}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
64 5—66 4	1	1	74 5	17	17	84 5	0	1
66 5	4	2	76 5	18	22	86 5	3	2
68 5	4	4	78 5	22	13	88 5		0
70 5	4	11	80 5	7	4	90 5		0
72 5	15	19	82 5	4	3	92 5		1
							100	100

	Female	Male	Difference	X
Mean and P E	76 6 ± 0 30	76 1 ± 0 29	+ 0 5 ± 0 42	
S D and P E	4 5 ± 0 21	4 4 ± 0 21	+ 0 1 ± 0 30	100 5
C V and P E	5 8 ± 0 28	5 7 ± 0 27	+ 0 1 ± 0 39	

Table XXVI

$$\text{Height breadth index} = \frac{\text{Auricular height} \times 100}{\text{Maximum head breadth}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
72 5—74 4	1	3	84 5	13	16	96 5	3	2
74 5	3	1	86 5	16	16	98 5	0	2
76 5	2	3	88 5	12	13	100 5	0	0
78 5	4	4	90 5	9	11	102 5	1	0
80 5	11	8	92 5	6	3	104 5		1
82 5	15	12	94 5	4	5		100	100

	Female	Male	Difference	X
Mean and P E	86 2 ± 0 36	87 0 ± 0 38	− 0 8 + 0 53	
S D and P E	5 4 ± 0 26	5 7 ± 0 27	− 0 3 + 0 37	99 1
C V and P E	6 2 ± 0 30	6 5 ± 0 31	− 0 3 + 0 43	

Table XXVII

$$\text{Cranial module} = \frac{\text{Maximum head length} + \text{maximum head breadth} + \text{auricular height}}{3}$$

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
133 5–135 4	1		147 5	28	6	161 5		1
135 5	0		149 5	11	16	163 5		0
137 5	4		151 5	6	18	165 5		0
139 5	5		153 5		25	167 5		0
141 5	14		155 5		15	169 5		1
143 5	15	1	157 5		9			
145 5	16	5	159 5		3		100	100

	Female	Male	Difference	X
Mean and P E	146 3 ± 0 25	153 7 ± 0 26	− 7 5 ± 0 36	
S D and P E	3 6 ± 0 17	3 8 ± 0 18	− 0 2 ± 0 25	95 2
C V and P E	2 5 ± 0 12	2 5 ± 0 12	0 0 ± 0 17	

Table XXVIII

Height vertex to sub-nasal

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
115–116 9		1	141	7	5	167	0	7
117		0	143	6	5	169	2	5
119		0	145	6	8	171	1	2
121		0	147	7	4	173	1	3
123		1	149	13	4	175	2	1
125	1	0	151	7	4	177	0	0
127	1	1	153	3	6	179	0	2
129	2	0	155	4	2	181	1	1
131	1	0	157	5	5	183	1	0
133	3	2	159	6	8	185		0
135	0	3	161	6	5	187		0
137	4	2	163	3	6	189		0
139	6	3	165	1	3	191		1
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	150 4 ± 0 77	154 8 ± 1 01	− 3 4 ± 1 32	
S D and P E (mms)	11 4 ± 0 55	15 0 ± 0 71	− 3 5 ± 0 90	97 1
C. V and P E (per cent)	7 6 ± 0 36	9 7 ± 0 47	− 2 1 ± 0 59	

Table XXIX

Physiognomic facial length

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
145–146 9	1		165	7	4	185	1	4
147	3		167	11	13	187		1
149	2		169	9	8	189		0
151	5		171	10	11	191		1
153	2	1	173	5	7	193		4
155	7	2	175	4	7	195		1
157	9	3	177	3	5	197		0
159	5	1	179	1	7	199		1
161	5	6	181	3	2			
163	6	7	183	1	4			
							100	100

	Female	Male	Difference	X
Mean and P F (mms)	164 8 ± 0 60	172 7 ± 0 64	− 7 9 ± 0 87	95 4
S D and P E (mms)	9 0 ± 0 13	9 6 ± 0 46	− 0 6 ± 0 62	
C V and P E (per cent)	5 5 ± 0 26	5 5 ± 0 27	− 0 1 ± 0 37	

Table XXX

$$\textit{Physiognomic facial index} = \frac{\textit{Bizygomatic breadth} \times 100}{\textit{Physiognomic facial length}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
63 5—65 4	1	1	73 5	16	13	83 5	4	4
65 5	2	2	75 5	23	14	85 5	1	1
67 5	2	8	77 5	15	17	87 5		1
69 5	7	5	79 5	11	8	89 5		0
71 5	8	14	81 5	10	11	91 5		1
							100	100

	Female	Male	Difference	X
Mean and P E	76 7 ± 0 29	76 4 ± 0 40	+ 0 3 ± 0 49	100 4
S D and P E	4 3 ± 0 20	5 9 ± 0 28	− 1 6 ± 0 35	
C V and P E	5 6 ± 0 27	7 7 ± 0 37	− 2 0 ± 0 45	

Table XXXI

Morphological facial length

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
91—92 9	1	1	107	8	9	123	1	1
93	8	1	109	12	8	125		3
95	3	2	111	10	7	127		0
97	5	1	113	3	8	129		1
99	12	1	115	3	7	131		0
101	9	9	117	2	11	133		1
103	12	7	119	3	6			
105	8	13	121	0	3		100	100

	Female	Male	Difference	X
Mean and P E (mms)	105 0 ± 0 48	110 6 ± 0 54	− 5 6 ± 0 72	94 9
S D and P E (mms)	6 9 ± 0 33	8 0 ± 0 38	− 1 0 ± 0 50	
C V and P E (per cent)	6 6 ± 0 32	7 2 ± 0 35	− 0 6 ± 0 47	

Table XXXII

$$\textit{Total facial index} = \frac{\textit{Morphological facial length} \times 100}{\textit{Bizygomatic breadth}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
67 5—69 4		1	81 5	20	12	95 5	2	2
69 5	1	2	83 5	8	14	97 5	0	0
71 5	3	1	85 5	13	13	99 5	0	0
73 5	4	3	87 5	6	4	101 5	1	1
75 5	7	10	89 5	5	8			
77 5	9	9	91 5	5	6		100	100
79 5	14	8	93 5	2	6			

	Female	Male	Difference	X
Mean and P E	83 4 ± 0 39	81 1 ± 0 42	+ 2 2 ± 0 57	102 7
S D and P E	5 8 ± 0 28	6 2 ± 0 30	− 0 4 ± 0 41	
C V and P E	7 0 ± 0 34	7 4 ± 0 35	− 0 4 ± 0 49	

TABLE XXXIII

Physiognomic superior facial length

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
61—62 9	2	1	71	14	11	81	1	3
63	1	2	73	13	14	83	1	2
65	14	11	75	9	16	85		1
67	11	13	77	2	9			
69	22	13	79	6	4		100	100

	Female	Male	Difference	X
Mean and P E (mms)	70 6 ± 0 31	72 3 ± 0 34	− 1 6 ± 0 46	
S D and P E (mms)	4 6 ± 0 22	5 0 ± 0 24	− 0 4 ± 0 32	97 7
C V and P E (per cent)	6 5 ± 0 31	6 9 ± 0 33	− 0 4 ± 0 45	

TABLE XXXIV

Morphological superior facial length

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
49—50 9	1	1	61	20	15	73		2
51	1	0	63	15	17	75		1
53	1	3	65	16	11	77		0
55	4	0	67	9	13	79		1
57	10	11	69	10	6			
59	10	17	71	3	2		100	100

	Female	Male	Difference	X
Mean and P E (mms)	62 9 ± 0 30	63 2 ± 0 33	− 0 2 ± 0 45	
S D and P E (mms)	4 5 ± 0 21	4 9 ± 0 23	− 0 4 ± 0 33	99 5
C V and P E (per cent)	7 1 ± 0 34	7 7 ± 0 37	− 0 6 ± 0 49	

Table XXXV

Superior facial index = $\frac{\text{Morphological superior facial length} \times 100}{\text{Bizygomatic breadth}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
36 5—38 4		1	46 5	12	20	56 5	4	0
38 5	1	2	48 5	20	23	58 5	1	0
40 5	1	3	50 5	20	14	60 5		1
42 5	6	13	52 5	10	7			
44 5	9	14	54 5	7	2		100	100

	Female	Male	Difference	X
Mean and P E	50 0 ± 0 25	48 0 ± 0 26	+ 2 0 ± 0 36	
S D and P E	3 7 ± 0 18	3 8 ± 0 18	− 0 1 ± 0 25	104 1
C V and P E	7 5 ± 0 36	7 9 ± 0 38	− 0 3 ± 0 52	

Table XXXVI

Nasal length in ground plan

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
40—41 9	4	5	48	17	19	56	1	5
42	11	12	50	13	19	58	1	
44	12	13	52	9	9			
46	25	15	54	7	3		100	100

	Female	Male	Difference	X
Mean and P E (mms)	47 8 ± 0 26	47 9 ± 0 27	− 0 1 ± 0 38	
S D and P E (mms)	3 8 ± 0 37	4 0 ± 0 19	− 0 2 ± 0 41	99 9
C V and P E (per cent)	8 0 ± 0 40	8 3 ± 0 40	− 0 3 ± 0 57	

Table XXXVII

$$\text{Nasal index} = \frac{\text{Nasal breadth} \times 100}{\text{Nasal length in ground plan}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
49 5—51 4	1		63 5	6	5	77 5	4	4
51 5	0		65 5	11	4	79 5	5	13
53 5	0		67 5	10	5	81 5	1	6
55 5	4	1	69 5	10	11	83 5	2	8
57 5	6	0	71 5	8	10	85 5	1	0
59 5	9	6	73 5	9	8	87 5		5
61 5	8	5	75 5	5	5	89 5		4
							100	100

	Female	Male	Difference	X
Mean and P E	68 7 ± 0 48	74 8 ± 0 57	− 6 1 ± 0 75	91 9
S D and P E	7 2 ± 0 34	8 5 ± 0 40	− 1 3 ± 0 53	
C V and P E	10 4 ± 0 50	11 3 ± 0 55	+ 9 3 ± 0 74	

Table XXXVIII

Nasal length along profile

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
43—44 9	2	7	53	14	12	63	1	3
45	5	9	55	11	16	65		1
47	20	9	57	6	8			
49	18	16	59	2	0			
51	19	18	61	2	1		100	100

	Female	Male	Difference	X
Mean and P E (mms)	51 4 ± 0 27	51 7 ± 0 31	− 0 2 ± 0 41	99 5
S D and P E (mms)	4 0 ± 0 19	4 8 ± 0 22	− 0 8 ± 0 29	
C V and P E (per cent)	7 8 ± 0 37	9 2 ± 0 44	− 1 5 ± 0 58	

Table XXXIX

Nasal height projection from face

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
15—16 9	7	3	21	11	41	27	1	
17	30	12	23	8	13			
19	43	25	25	0	6		100	100

	Female	Male	Difference	λ
Mean and P E (mms)	18 3 ± 0 14	20 8 ± 0 15	− 2 6 ± 0 20	
S D and P E (mms)	2 1 ± 0 10	2 2 ± 0 10	− 0 1 ± 0 15	87 5
C V and P E (per cent)	11 4 ± 0 56	10 4 ± 0 50	− 1 0 ± 0 75	

Table XL

Frontal height, physiognomic

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
42—43 9	2		58	12	10	74	0	4
44	3		60	3	9	76	2	0
46	2	1	62	13	7	78		1
48	5	1	64	6	10	80		1
50	6	1	66	4	12	82		2
52	10	4	68	7	9	84		2
54	11	13	70	5	6			
56	8	6	72	1	1		100	100

	Female	Male	Difference	X
Mean and P E (mms)	58 4 ± 0 51	63 2 ± 0 53	− 4 8 ± 0 73	
S D and P E (mms)	7 6 ± 0 36	7 8 ± 0 37	− 0 2 ± 0 52	92 3
C V and P E (per cent)	13 0 ± 0 63	12 5 ± 0 61	+ 0 5 ± 0 88	

TABLE XLI

Height of mucous lips

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
9—10 9		1	17	33	19	25	1	3
11	3	1	19	30	30	27		1
13	5	4	21	8	14			
15	17	8	23	3	11		100	92

	Female	Male	Difference	X
Mean and P E (mms)	18 0 ± 0 17	19 4 ± 0 22	− 1 4 ± 0 27	
S D and P E (mms)	2 5 ± 0 12	3 1 ± 0 15	− 0 6 ± 0 19	92 7
C V and P E (per cent)	13 9 ± 0 67	15 8 ± 0 80	− 1 9 ± 1 05	

TABLE XLII

Height of entire upper lip

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
14—15 9		2	20	34	29	26		6
16	8	3	22	17	28			
18	39	19	24	2	13		100	100

	Female	Male	Difference	X
Mean and P E (mms)	19 8 ± 0 12	21 3 ± 0 17	− 1 5 ± 0 21	
S D and P E (mms)	1 7 ± 0 07	2 5 ± 0 12	− 0 8 ± 0 14	93
C V and P E (per cent)	8 8 ± 0 42	11 8 ± 0 49	− 3 0 ± 0 64	

TABLE XLIII

Height of entire lower lip

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
10—11 9	1	2	20	9	28	30		0
12	7	4	22	2	16	32		0
14	27	12	24		4	34		0
16	34	17	26		3	36		1
18	20	12	28		1		100	100

	Female	Male	Difference	X
Mean and P E (mms)	16 5 ± 0 15	19 2 ± 0 27	− 2 7 ± 0 31	8 56
S D and P E (mms)	2 3 ± 0 11	4 0 ± 0 19	− 1 7 ± 0 22	
C V and P E (per cent)	13 8 ± 0 67	20 3 ± 1 01	− 6 5 ± 1 27	

TABLE XLIV

Height of chin

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
30—31 9	1		42	11	15	54		3
32	3	1	44	22	15	56		0
34	3	1	46	6	11	58		2
36	13	5	48	1	11		100	100
38	18	8	50	4	10			
40	18	15	52		3			

	Female	Male	Difference	X
Mean and P E (mms)	41 0 ± 0 28	44 6 ± 0 34	− 3 5 ± 0 44	90
S D and P E (mms)	4 1 ± 0 20	5 1 ± 0 24	− 1 0 ± 0 31	
C V and P E (per cent)	10 0 ± 0 48	11 4 ± 0 55	− 1 4 ± 0 73	

TABLE XLV

Physiognomic ear length

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
36—37 9	1		50	6		64	2	11
38	0		52	12		66	0	5
40	0		54	16	3	68	0	2
42	0		56	22	10	70	0	2
44	0		58	20	23	72	1	
46	0		60	15	29			
48	1		62	4	15		100	100

	Female	Male	Difference	X
Mean and P E (mms)	56 6 ± 0 28	60 9 ± 0 22	− 4 2 ± 0 36	
S D and P E (mms)	4 2 ± 0 20	3 3 ± 0 16	+ 0 9 ± 0 25	93 1
C V and P E (per cent)	7 4 ± 0 36	5 3 ± 0 26	+ 2 1 ± 0 44	

TABLE XLVI

Physiognomic ear breadth

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
24—25 9	1		32	36	19	40	0	5
26	3		34	24	30	42	0	1
28	10		36	7	23	44	1	
30	18	12	38	0	10			
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	32 4 ± 0 18	34 9 ± 0 18	− 2 5 ± 0 26	
S D and P E (mms)	2 7 ± 0 13	2 7 ± 0 13	+ 0 0 ± 0 18	92 6
C V and P E (per cent)	8 6 ± 0 41	7 7 ± 0 37	+ 0 9 ± 0 55	

Table XLVII

Physiognomic aural index $= \frac{\text{Physiognomic ear breadth} \times 100}{\text{Physiognomic ear length}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
40 5—42 4	1	.	52 5	13	7	64 5	2	1
42 5	0	.	54 5	12	14	66 5	1	.
44 5	0		56 5	17	19	68 5	1	
46 5	5		58 5	19	19			
48 5	3	3	60 5	13	18		100	100
50 5	7	13	62 5	6	6			

	Female	Male	Difference	X
Mean and P E	57 1 ± 0 21	57 5 ± 0 26	− 0 4 ± 0 33	
S D and P E	3 1 ± 0 15	3 8 ± 0 18	− 0 7 ± 0 23	0 9 4
C V and P E	5 5 ± 0 26	6 2 ± 0 32	− 0 7 ± 0 41	

Table XLVIII

Morphological ear length

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
17—18 9	1		25	30	11	33	1	11
19	2		27	32	27	35	1	4
21	5		29	7	28			
23	16	4	31	5	15		100	100

	Female	Male	Difference	X
Mean and P E (mms)	26 4 ± 0 16	29 3 ± 0 18	− 2 9 ± 0 24	
S D and P E (mms)	2 4 ± 0 12	2 8 ± 0 13	− 0 4 ± 0 18	8 9 9
C V and P E (per cent)	9 2 ± 0 44	9 5 ± 0 45	+ 0 3 ± 0 63	

TABLE XLIX

Morphological ear breadth

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
35—36 9		1	47	11	26	59	0	
37	3	0	49	8	21	61	0	
39	5	0	51	6	12	63	1	
41	16	2	53	5	12			
43	23	10	55	1	5			
45	21	10	57	0	1		100	100

	Female	Male	Difference	X
Mean and P E (mms)	45 4 ± 0 29	48 8 ± 0 25	− 3 3 ± 0 38	93
S D and P E (mms)	4 3 ± 0 20	3 7 ± 0 18	+ 0 5 ± 0 27	
C V and P E (per cent)	9 4 ± 0 45	7 6 ± 0 37	+ 1 8 ± 0 58	

TABLE L

Morphological aural index = $\frac{\text{Morphological ear breadth} \times 100}{\text{Morphological ear length}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
105 5–110 4		1	160 5	8	15	215 5	1	0
110 5	1	1	165 5	8	5	220 5	2	1
115 5	0	0	170 5	9	6	225 5	0	
120 5	0	0	175 5	11	10	230 5	0	
125 5	1	0	180 5	12	12	235 5	0	
130 5	1	4	185 5	5	6	240 5	1	
135 5	0	3	190 5	0	2	245 5	1	
140 5	2	1	195 5	5	3	250 5	1	
145 5	10	6	200 5	3	2			
150 5	6	5	205 5	1	1			
155 5	8	15	210 5	3	1		100	100

	Female	Male	Difference	X
Mean and P E	173 7 ± 1 63	167 4 ± 1 34	+ 6 3 ± 2 1	103 5
S D and P E	24 1 ± 1 15	19 9 ± 0 95	+ 4 3 ± 1 49	
C V and P E	13 9 ± 0 68	11 9 ± 0 57	+ 2 0 ± 0 89	

Table LI

Horizontal circumference of the head

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
181—185 9	1		521	6	1	561		4
486	2		526	20	1	566		3
191	1		531	10	13	571		3
196	4		536	9	9	576		2
501	2		541	4	12	581		2
506	11		546	3	10			
511	10		551	2	20			
516	15		556		11		100	99

	Female	Male	Difference	Y
Mean and P E (mms)	521 8 ± 0 97	549 1 ± 0 95	— 27 3 ± 0 43	95
S D and P E (mms)	14 4 ± 0 69	14 0 ± 0 67	+ 0 4 ± 0 91	
C V and P E (per cent)	2 8 ± 0 13	2 5 ± 0 12	+ 0 2 ± 0 18	

Table LII

Sagittal arc of the head

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
286—290 9	1		331	13	6	376		2
291	0		336	9	9	381		1
296	3		341	6	13	386		0
301	2		346	2	13	391		2
306	5		351	1	16	396		0
311	6	2	356	1	7	401		0
316	19	3	361		7	406		1
321	17	1	366		7			
326	15	9	371		0		100	99

	Female	Male	Difference	X
Mean and P E (mms)	324 5 ± 0 55	347 8 ± 1 04	— 23 4 ± 1 18	93 5
S D and P E (mms)	8 2 ± 0 39	15 3 ± 0 73	— 7 1 ± 0 77	
C V and P E (per cent)	2 5 ± 0 12	4 4 ± 0 21	— 1 9 ± 0 24	

TABLE LIII

Sagittal cranial curvature index = $\frac{\text{Glabella inion length} \times 100}{\text{Sagittal arc}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
46 5—48 4	2	5	54 5	30	12	62 5		0
48 5	3	7	56 5	10	10	64 5		1
50 5	21	22	58 5	1	1			
52 5	33	41	60 5		0		100	99

	Female	Male	Difference	X
Mean and P E	53 9 ± 0 18	53 3 ± 0 17	+ 0 6 ± 0 25	
S D and P E	2 2 ± 0 13	2 6 ± 0 12	− 0 4 ± 0 18	101 1
C V and P E	4 1 ± 0 19	4 8 ± 0 23	− 0 8 ± 0 30	

TABLE LIV

Transverse arc of the head

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
301—305 9	1		336	13	10	371		5
306	0		341	16	13	376		0
311	2		346	17	11	381		3
316	4		351	3	14	386		3
321	9	3	356	4	7			
326	12	3	361	1	8		100	99
331	18	9	366		10			

	Female	Male	Difference	X
Mean and P E (mms)	336 4 ± 0 75	351 3 ± 1 04	− 14 9 ± 1 55	
S D and P E (mms)	11 1 ± 0 53	15 3 ± 0 73	− 4 2 ± 0 91	95 7
C V and P E (per cent)	3 3 ± 0 16	4 4 ± 0 21	− 1 1 ± 0 26	

TABLE LV

Transverse cranial curvature index = $\frac{\text{Biauricular breadth} \times 100}{\text{Transverse arc}}$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
29 5—31 4		1	33 5	25	18	37 5	23	28
31 5	6	5	35 5	42	42	39 5	4	5
							100	99

	Female	Male	Difference	λ
Mean and P E	36 4 ± 0 12	36 6 ± 0 12	− 0 3 ± 0 18	
S D and P E	1 8 ± 0 08	1 9 ± 0 09	− 0 1 ± 0 34	0 91
C V and P E	4 9 ± 0 23	5 1 ± 0 25	− 0 2 ± 0 12	

(*b*) SUMMARY AND DISCUSSIONS OF THE HEAD MEASUREMENTS

Measurements of the male group

In the absence of suitable material to compare with the results of the present study, comparisons are few Under the Ethnological Society of India (now extinct) some measurements of individuals of different castes in India were taken in 1906 The report about the Bombay Presidency is published in 1907 The book does not

mention any methods employed in taking measurements A few comparisons which can be elicited are as follows —

In the report the highest cranial length is 186 4 mm and of Konkanastha Brahmins (100) Average cranial length of our cosmopolitan group is 187 9 mm (Table V) The head width from our study is 146 36 mm (Table VII) and approaches the standard of Saraswat Brahmins (100) from the report Our bizygomatic breadth (Table XI) is 131 7 mm and is the same for Mahars from the report (for summary of the report *see* pp 340-41)

The average cranial index of our study is 77 92 per cent (Table VIII), showing the head to be mesocephalic (mesocephalic range 76–81) The least frontal breadth is 102 5 mm (Table IX) The transverse fronto-parietal index is 70 2 per cent (Table X) indicating the head and face to be eurymetopic (69) The zygomatico-frontal index is 77 32 per cent (Table XII), bigonial breadth is 89 mm (Table XIV) and the zygomatico-mandibular index is 67 9 per cent (p 19) Auricular and bimastoid breadths are 128 38 and 128 48 mm , showing the same figures (Tables XVI and XVII) Biocular and interocular breadths are 92 52 and 30 8 mm (Tables XVIII and XIX), while interpupillo-cranial index is 42 24 per cent (Table XX) Nasal and oral breadths are 35 64 and 48 3 mm (Tables XXI and XXII) Auricular height indicating the cranial height is 127 02 mm (Table XXIII) Length auricular height index is 67 5 per cent (Table XXIV) showing the average head to be hypsicephalic (75), while the mean height index is 76 14 per cent (Table XXV) Height breadth index is 86 96 per cent (Table XXVI) and the cranial module is 153 72 mm (Table XXVII) Height vertex to sub-nasal is 154 8 mm (Table XXVIII) Physiognomic facial length is 172 66 mm (Table XXIX) Physiognomic facial index is 76 44 per cent (Table XXX) Morphological facial length is 110 6 mm (Table XXXI) and the total facial index is 81 14 per cent (Table XXXII) indicating the face to be euryprosopic (80–85) Physiognomic superior facial length is 72 26 mm (Table XXXIII) while morphological one is 63 16 mm (Table XXXIV) Superior facial index is 48 per cent (Table XXXV) and so it is euryne (45–50) Nasal length in ground plan is 47 88 mm (Table XXXVI) The nasal index is 74 78 per cent (Table XXXVII) and the nose is mesorhinic (on head 70–84 9 and on skull 48–52 9) Nasal length along profile is 51 68 mm (Table XXXVIII) Nose salient is 20 84 mm (Table XXXIX) Frontal height is 63 16 mm (Table XL) Heights of mucous lips, entire upper and lower lips are 19 36, 21 32 and 19 24 mm respectively (Tables XLI, XLII and XLIII) Height of the chin is 44 58 mm (Table XLIV) Physiognomic ear lengths and breadths are 60 86 and 34 88 mm (Tables XLV, and XLVI) Physiognomic aural index is 57 46 (Table XLVII) Morphological ear lengths and breadths are 29 26 and 48 78 mm (Tables XLVIII and XLIX) and the morphological ear index is 167 4 per cent (Table L) Horizontal circumference of the head is 549 mm (Table LI) Sagittal arc 347 8 (Table LII) and the

transverse arc 351 3 mm (Table LIV) Sagittal cranial and transverse cranial curvature indices are 53 29 per cent and 36 64 per cent (Tables LIII and LV) respectively

The relations of the deviations of subjects about the standard vary widely according to the magnitude in case of measurements Smaller the measurement greater is the variation, and greater the measurement smaller in proportion is the variation The least variation (2 52 per cent) is about the horizontal circumference the head (Table LI) The greatest variation (20 3 per cent) has been found to about the height of the entire lower lip (Table XLIII) being the measurement of the least magnitude in the head

The variation in head indices ranges from 2 48 to 11 86 per cent for cranial module (Table XXVII) and morphological aural index (Table L)

Measurements of the female group

The measurements of the female group cannot be compared with any material The figures for American women which were available to us from the *Journal of Physical Anthropology*, Vol XIII (p 189) (Anthropometry at Smith College by M Steggerda, J Crane, and M D Steele) have been however compared with the present study

Maximum head length of our group is 176 36 mm (Table V), while the figures from different studies in America are 186 43, 186 88, and 180 22 mm Head width is 140 46 (Table VII) and American groups have 145 98, 147 11, and 145 22 mm Our cephalic index is 80 per cent (Table VIII) showing the head to be mesocephalic The cephalic indices of American groups are 78 5, 78 96, 80 22, all meso cephalic The least frontal breadth is 100 36 mm (Table IX), and American groups have 117 5 and 102 7 mm (the author of the American study has expressed the doubt about the correctness of method applied in taking this measurement, ...o to a vast difference in two groups there) Fronto-parietal index of our group ...er cent (Table X) and the Americans have 80 49 and 76 49 The head of C V and ... eurymetopic Our bizygomatic breadth is 125 96 mm (Table XI) are 130 46 and 116 35 mm Our zygomatico-frontal index is ——— XII) Transverse cranio-facial index is 89 76 (Table XIII) ...4 mm (Table XIV) American bigonial breadths are

(*b*) SUMMARY A Our zygomatico-mandibular index is 65 76 per cent and bimastoid breadths are 122 36 and 121 92 mm ...and XVII) Biocular and interocular breadths are 90 44

In the absence of surely (Tables XVIII and XIX) Interpupillo-cranial index study, comparisons are few XX) which, it is amusing to note, is nearly the same some measurements of imps (43 07 and 43 36) Nasal and oral breadths are 32 64 The report about the Bom XXI and XXII)

Those of Americans are 32 28 and 49 51 mm Our auricular height is 125 74 mm (Table XXIII) and the American studies give 124 45, 128 14 and 112 42 mm as theirs Our length auricular height index is 69 14 per cent (Table XXIV) showing the head to be hypsicephalic The American figures are 66 59, 68 7 and 68 18 Our mean height index is 76 62 per cent (Table XXV) Height breadth index is 86 2 per cent (Table XXVI) American index is 84 89 and 85 86 The cranial module of our group is 146 26 (Table XXVII) Height vertex to sub-nasal is 150 44 (Table XXVIII) Physiognomic facial length is 164 8 mm (Table XXIX) and physiognomic index is 76 72 per cent (Table XXX) American physiognomic facial lengths are 171 91 and 175 59 mm Our morphological facial length is 105 mm (Table XXXI) and Americans have 111 99 mm Our total facial index is 83 36 per cent (Table XXXII) while that of Americans is 86 95 and 90 40 , the head of our group is euryprosopic Physiognomic superior facial length is 70 52 mm (Table XXXIII), while morphological one is 62 94 mm (Table XXXIV) Superior facial index is 50 per cent (Table XXXV) and the face is euryene Nasal length in ground plan is 47 76 mm (Table XXXVI) and Americans have 50 51, 53 61 and 49 62 mm as their figures Our nasal index is 68 68 per cent (Table XXXVII) and the nose is leptorhinic Figures for Americans are 63 71, 59 89 and 66 17 Our nasal length along profile is 51 44 mm (Table XXXVIII) Nasal height is 18 29 mm (Table XXXIX) American nose salient is 19 31 mm Our frontal height is 58 4 mm (Table XL) Heights of mucous lips and entire upper and lower lips are 17 96 19 82, 16 5 mm respectively (Tables XLI, XLII and XLIII) Height of the chin is 41 04 (Table XLIV) Physiognomic ear length and breadth are 56 62 and 32 36 mm (Tables XLV and XLVI) The American figures are 58 87, 58 89 and 58 98 mm for length and 33 23, 30 99 and 30 18 mm for breadth Our physiognomic aural index is 57 06 per cent (Table XLVII) and Americans' are 56 31, 50 63 and 51 30 mm Our morphological ear length and breadth are 26 38 and 45 44 mm (Tables XLVIII and XLIX) Morphological aural index is 173 65 per cent (Table L) Horizontal circumference of the head is 521 8 mm (Table LI) and Americans have 550 15 mm Our sagittal arc is 324 45 mm (Table LII) Sagittal cranial curvature index is 53 9 per cent (Table LIII) Transverse arc of the head is 336 4 mm (Table LIV) Americans having 336 12 mm Our transverse cranial curvature index is 36 38 per cent (Table LV)

The relation of the deviation of subjects from the standard varies according to the magnitude of the measurement as in the male group The least variation 2 53 per cent in case of a female group is about the sagittal arc (Table LII) and the highest 13 87 per cent about the height of the mucous lips (Table XLI) Least and the highest variations of the male group were about the entire lower lip (Table XLIII) and the horizontal circumference of the head (Table LI)

The variation about the standards of cephalic indices ranges from 2 49 to 13 9 per cent for cranial module (Table XXVII) and morphological aural index as in the male group (Table L)

Comparison of male and female standards (Fig 1)

Comparison of standards of two groups of different sex is interesting Three head measurements of the female group, morphological superior facial length, nasal length in ground plan and nasal length along profile (Tables XXXIV, XXXVI and XXXVIII) have nearly approached the male standards (more than 99 per cent) Auricular height (Table XXIII) is 98 8 per cent of the male Frontal and biocular breadth, height vertex to sub-nasal and physiognomic facial lengths (Tables IX, XVIII, XXVIII, XXXIII) are 97-98 per cent Head width, bizygomatic breadth auricular breadth, interocular breadth, physiognomic facial length, horizontal circumference of the head and the transverse arc of the head are 95-96 per cent (Tables VII XI XVI, XIX, XXIX, LI and LIV) Glabella-Inion length, bimastoid breadth, oral breadth and morphological facial lengths are 94-95 per cent (Tables VI, XVII, XXII and XXXI) Greatest cranial length, height of the entire upper lip, physiognomic ear length, morphological ear breadth, and sagittal arc of the head are 93-94 per cent (Tables V, XLII, XLV, XLIX and LII) Bigonial breadth, frontal height, height of the mucous lips and physiognomic ear breadth are 92-93 per cent (Tables XIV, XL, XLI and XLVI) Nasal breadth (Table XXI) is 92 per cent Height of the chin (Table XLIV) is 90 per cent Morphological ear length (Table XLVIII) is 89 per cent Nose salient (Table XXXIX) is 87 per cent Height of the entire lower lip (Table XLIII) is 85 86 per cent

The aggregate average proportion of the female head to the male head is 94 4 per cent , the individual standards ranging widely from 85–99 per cent (*see* Fig 1)

Comparison of indices shows that transverse cranio-facial and superior facial indices (Tables XIII and XXXV) are more than 104 per cent of the same of the male group Zygomatic frontal and morphological aural indices (Tables XII and L) are more than 103 per cent Cephalic interpupillo-cranial, length auricular height and total facial indices are more than 102 per cent (Tables VIII, XX XXIV and XXXII) Transverse fronto-parietal and sagittal cranial curvature indices (Tables X and LIII) are more than 101 per cent Mean height and physiognomic facial indices are more than 100 per cent (Tables XXV and XXX) Height breadth, physiognomic aural, and transverse cranial curvature indices are more than 9[illegible] per cent (Tables XXVI, XLVII and LV) Cranial module (Table XXVII) is more than 95 per cent Zygomatico-mandibular and nasal indices (Tables XV and XXXVII) are more than 91 per cent

The aggregate average of indices of the female head is 100 38 per cent of the male aggregate

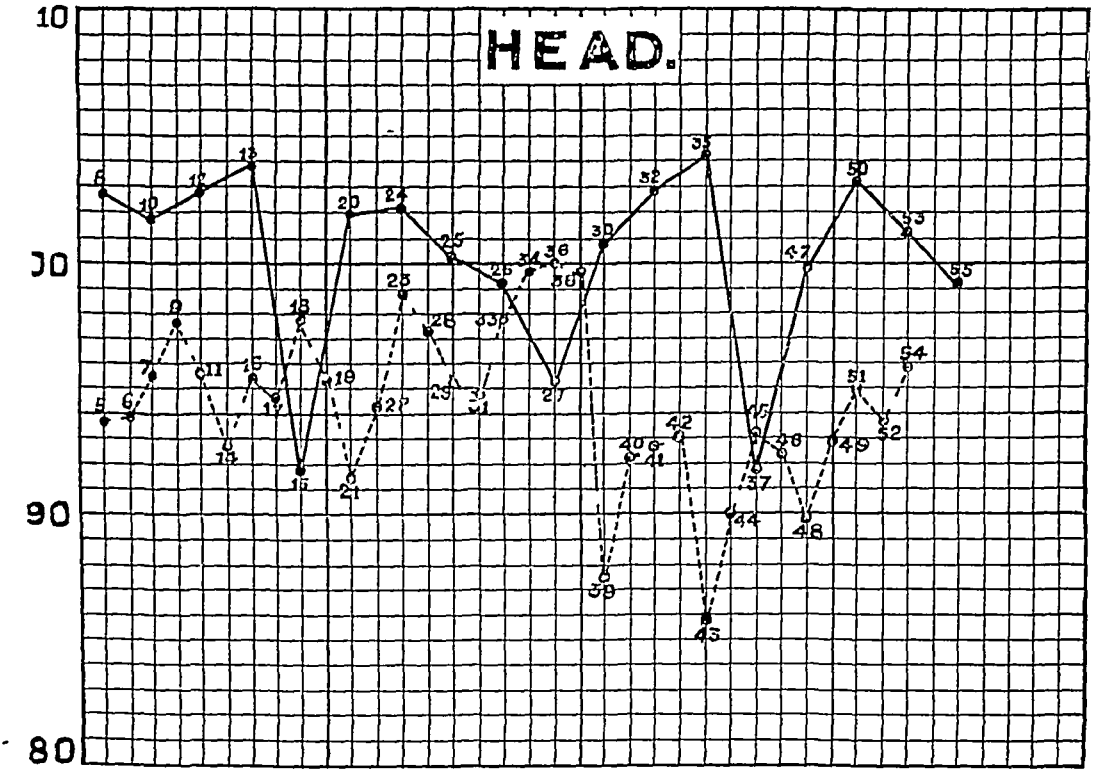

FIG 1

Comparison of head study of males and females

A graph showing the comparison of the standards of head measurements and indices The standards of the female measurements and indices have been reduced to the percentage of the male standards Thus all male standards represent the line 100 The numbered dots along the broken line indicate the table numbers of measurements of female group and those along the unbroken line indicate indices of the same The numbers refer to table numbers

Dot numbers along the broken line (measurements)

5 Max Head L
6 Glab Inion L
7 Max Head B
9 Lst. Front B
11 Bizygom B
14 Bigonial B
16 Auricular B
17 Bimastoid B
18 Biocular B

19 Interocul B
21 Nasal B
22 Oral B
23 Auricular Ht
28 Ht Vt Sub nas
29 Phys Fac Length
31 Morp Fac Length
33 Phys Sup Fac Length
34 Morp Sup Fac Length

36 Nas L gr plan
38 Nas L profile
39 Nas height
40 Front ht phys
41 Ht mucous lips
42 Ht entire up lip
43 Ht entire lower lip
44 Ht of chin
45 Phys ear length

46 Phys ear breadth
48 Morp ear length
49 Morp ear breadth
51 Hor Head circ
52 Sag arc head
54 Trans arc head

Dot numbers along the unbroken line (indices)

8 $\frac{\text{Head breadth}}{\text{Head length}}$
10 $\frac{\text{L t front B}}{\text{Head width}}$
12 $\frac{\text{Lst. Front B}}{\text{Bizygom B}}$
13 $\frac{\text{Bizygom B}}{\text{Max. head B}}$
15 $\frac{\text{Bigonal B}}{\text{Bizygom B}}$

20 $\frac{\text{Bioc B + Interoc B}}{\text{2 Max head breadth}}$
24 $\frac{\text{Auricular height}}{\text{Max head length}}$
25 $\frac{\text{Auricular ht.}}{\text{Mn of Max head L + B}}$
26 $\frac{\text{Auricular ht}}{\text{Max. head breadth}}$
27 $\frac{\text{Max head L + B + Auric Ht}}{3}$

30 $\frac{\text{Bizygomatic B}}{\text{Phys fac length}}$
32 $\frac{\text{Morp fac length}}{\text{Bizygom. breadth}}$
35 $\frac{\text{Morp sup fac L}}{\text{Bizygom breadth}}$
37 $\frac{\text{Nasal breadth}}{\text{Nasal L gr plan}}$
47 $\frac{\text{Phys ear breadth}}{\text{Phys ear length}}$

50 $\frac{\text{Morp ear B}}{\text{Morp ear length}}$
53 $\frac{\text{Glab Inion L}}{\text{Sagittal arc}}$
55 $\frac{\text{Biauricular B}}{\text{Trans arc}}$

Both male and female heads are mesocephalic, eurymetopic and hypsicephalic Both male and female faces are euryprosopic and euryenes. The male nose is mesorhinic and the female one is leptorhinic

Study of Trunk.

(*a*) TABLES OF MEASUREMENTS AND INDICES OF TRUNK

TABLE LVI

Total stature

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
1391—00 9	1		1561	3	2	1731		1
1401	1		1571	3	4	1741		3
1411	3		1581	7	2	1751		4
1421	4		1591	4	4	1761		3
1431	3		1601	3	7	1771		0
1441	2		1611	1	5	1781		0
1451	5		1621	0	5	1791		1
1461	5		1631	2	8	1801		2
1471	5		1641	0	3	1811		0
1481	5		1651	0	3	1821		0
1491	5	1	1661	0	6	1831		0
1501	5	0	1671	0	7	1841		0
1511	4	0	1681	1	6	1851		0
1521	7	0	1691		3	1861		1
1531	6	1	1701		9			
1541	10	1	1711		3			
1551	5	3	1721		2		100	100

	Female	Male	Difference	X
Mean and P E (mms)	1518 4 ± 4 00	1660 9 ± 4 75	−142 5 ± 6 21	
S D and P E (mms)	59 3 ± 2 83	70 5 ± 3 36	− 11 3 ± 4 40	91 4
C V and P E (per cent)	3 9 ± 0 19	4 2 ± 0 20	− 0 3 ± 0 28	

TABLE LVII

Weights

Class kg	Z Female	Z Male	Class kg	Z Female	Z Male	Class kg	Z Female	Z Male
31—35	3		46	20	22	61	0	0
36	14	3	51	13	35	66	2	2
41	39	8	56	8	19	71	1	
							100	98

	Female	Male	Difference	X
Mean and P E (kg)	45 8 ± 0 48	52 3 ± 0 42	− 6 5 ± 0 64	
S D and P E (kg)	7 1 ± 0 33	6 1 ± 0 29	+ 1 0 ± 0 44	87 5
C V and P E (per cent)	15 6 ± 0 76	11 7 ± 0 60	+ 3 9 ± 0 97	

TABLE LVIII

Weight in grammes / Stature in centimetres

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
225—34 9	1		315	2	11	405	0	1
235	2		325	4	10	415	0	
245	3	2	335	5	9	425	0	
255	8	1	345	4	10	435	1	
265	9	8	355	4	3	445	0	
275	14	7	365	3	4	455	0	
285	11	7	375	2	1	465	1	
295	17	8	385	0	1	475	1	
305	8	14	395	0	1		100	98

	Female	Male	Difference	X
Mean and P E	304 2 ± 2 44	318 3 ± 2 21	− 14 1 ± 3 28	
S D and P E	36 1 ± 1 72	32 1 ± 1 56	− 4 2 ± 2 32	95 4
C V and P E	11 9 ± 0 61	10 1 ± 0 49	− 1 8 ± 0 79	

Table LIX

$$\text{Constitutional index} = \frac{\sqrt{\text{Weight in grammes}}}{\text{Total stature in centimetres}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
20 5—21 4		10	23 5	28	13	25 5	1	
21 5	18	39	24 5	16	2	26 5	3	
22 5	31	31					100	100

	Female	Male	Difference	λ
Mean and P E	23 6 ± 0 08	22 6 ± 0 06	+ 1 ± 0 10	1044
S D and P E	1 1 ± 0 05	0 9 ± 0 04	+ 0 3 ± 0 69	
C V and P E	4 8 ± 0 23	3 8 ± 0 18	+ 1 0 ± 0 29	

Table LX

Girth of thorax plane II (males only)

Class mms	Z Male	Class mms	Z Male	Class mms	Z Male	Class mms	Z Male
716—20 9	1	776	4	836	4	896	1
721	3	781	2	841	3	901	0
726	0	786	4	846	5	906	1
731	2	791	1	851	2	911	1
736	2	796	5	856	1	916	0
741	3	801	4	861	3	921	1
746	3	806	5	866	2	926	0
751	0	811	5	871	3	931	0
756	3	816	5	876	0	936	1
761	3	821	3	881	0		
766	1	826	3	886	1		100
771	2	831	4	891	1		

Mean and P E (mms)	805 5 ± 3 73
S D and P E (mms)	54 8 ± 2 64
C V and P E (per cent)	6 8 ± 0 33

TABLE LXI

Total stature minus (maximum thoracic girth + weight in kg)——males only

Class Fig	Z Male	Class Fig	Z Male	Class Fig	Z Male	Class Fig	Z Male
0—4 9	1	15	7	30	20	45	5
5	2	20	13	35	19	50	2
10	0	25	12	40	16		97

Mean and P E (Fig)	32 4 ± 0 67
S D and P E (Fig)	9 7 ± 0 47
C V and P E (per cent)	30 0 ± 1 57

TABLE LXII

Girth of thorax plane I (males only)

Class mms	Z Male	Class mms	Z Male	Class mms	Z Male	Class mms	Z Male
671—75 9	1	721	8	771	8	821	4
676	0	726	2	776	4	826	2
681	2	731	5	781	0	831	3
686	2	736	4	786	5	836	2
691	1	741	2	791	4	841	2
696	1	746	2	796	5	846	0
701	1	751	2	801	0	851	0
706	3	756	5	806	3	856	1
711	2	761	4	811	0		
716	0	766	4	816	4		98

Mean and P E (mms)	760 5 ± 3 89
S D and P E (mms)	57 2 ± 2 75
C V and P E (per cent)	7 5 ± 0 36

TABLE LXIII

Girth at waist (lowest girth of the trunk)——males only

Class mms	Z Male	Class mms	Z Male	Class mms	Z Male	Class mms	Z Male
536—40 9	1	601	2	666	4	731	0
541	0	606	2	671	5	736	0
546	1	611	2	676	5	741	0
551	0	616	1	681	3	746	1
556	1	621	6	686	3	751	0
561	1	626	5	691	4	756	0
566	1	631	4	696	0	761	0
571	0	636	6	701	1	766	2
576	0	641	3	706	5	771	1
581	2	646	3	711	3		
586	1	651	2	716	3		
591	0	656	6	721	4		
596	0	661	0	726	1		98

Mean and P E (mms)	662 5 ± 2 20
S D and P E (mms)	32 4 ± 1 56
C V and P E (per cent)	4 9 ± 0 24

TABLE LXIV

Tragion height

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
1281—90 9	2		1441	7	2	1601		2
1291	0		1451	1	3	1611		0
1301	5		1461	5	4	1621		5
1311	2		1471	6	5	1631		3
1321	4	1	1481	4	4	1641		2
1331	6	0	1491	1	6	1651		1
1341	1	0	1501	1	5	1661		0
1351	7	0	1511	2	6	1671		1
1361	5	0	1521	0	5	1681		0
1371	7	1	1531	0	7	1691		1
1381	4	0	1541	0	5	1701		0
1391	4	0	1551	1	7	1711		0
1401	6	1	1561		4	1721		1
1411	7	2	1571		5			
1421	5	0	1581		6		100	100
1431	7	2	1591		3			

	Female	Male	Difference	X
Mean and P E (mms)	1405 6 ± 3 98	1535 6 ± 5 37	— 130 0 ± 6 68	91 5
S D and P E (mms)	59 0 ± 2 82	79 6 ± 3 80	— 20 6 ± 4 73	
C V and P E (per cent)	4 2 ± 0 20	5 2 ± 2 48	— 1 0 ± 2 48	

TABLE LXV

Gnathion height

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
1191—00 9	1		1361	6	2	1531		3
1201	2		1371	2	5	1541		2
1211	1		1381	5	7	1551		3
1221	3		1391	6	5	1561		1
1231	5		1401	0	4	1571		1
1241	7		1411	3	8	1581		3
1251	4		1421	0	4	1591		0
1261	3		1431	0	5	1601		0
1271	7		1441	0	6	1611		0
1281	4	1	1451	1	4	1621		0
1291	7	0	1461		4	1631		0
1301	4	0	1471		6	1641		0
1311	4	0	1481		6	1651		0
1321	8	2	1491		5	1661		1
1331	5	0	1501		2			
1341	5	1	1511		3			
1351	7	2	1521		4		100	100

	Female	Male	Difference	X
Mean and P E (mms)	1312 8 ± 3 82	1455 9 ± 4 30	− 143 1 ± 5 75	
S D and P E (mms)	57 5 ± 2 70	64 4 ± 3 07	− 6 9 ± 4 12	90 2
C V and P E (per cent)	4 4 ± 0 21	4 5 ± 0 21	− 0 1 ± 0 3	

TABLE LXX

Height, Symphysion (males only)

Class mms	Z Male	Class mms	Z Male	Class mms	Z Male	Class mms	Z Male
761—70 9	2	821	9	881	5	941	1
771	3	831	9	891	1	951	1
781	3	841	5	901	6	961	2
791	4	851	6	911	6	971	0
801	2	861	10	921	2	981	1
811	4	871	9	931	4		
							98

Mean and P E (mms)	862 0 ± 3 26
S D and P E (mms)	47 9 ± 2 31
C V and P E (per cent)	5 6 ± 0 27

TABLE LXXI

Height, Iho-cristal

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
801—10 9	1		931	9	2	1051		4
811	0		941	6	4	1061		3
821	1		951	7	4	1071		4
831	2		961	7	2	1081		2
841	3		971	3	10	1091		4
851	4		981	1	3	1101		1
861	5		991	3	8	1111		0
871	10		1001	1	9	1121		2
881	2		1011	1	4	1131		0
891	6		1021	1	4	1141		0
901	12	2	1031		8	1151		0
911	7	1	1041		10	1161		1
921	8	8						
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	917 5 ± 3 13	1009 6 ± 3 95	— 92 1 ± 5 4	90 8
S D and P E (mms)	46 3 ± 2 21	58 6 ± 2 80	— 12 3 ± 3 56	
C V and P E (per cent)	5 0 ± 0 24	5 8 ± 0 28	— 0 8 ± 0 40	

Table LXXII

Height, Ilio-spinal

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
741—50 9	1		861	5	1	981	0	2
751	1		871	3	6	991	1	5
761	0		881	11	6	1001		3
771	2		891	9	4	1011		3
781	2		901	6	4	1021		2
791	6		911	3	8	1031		1
801	2		921	3	8	1041		0
811	9		931	1	8	1051		2
821	8		941	2	7	1061		0
831	9		951	0	7	1071		0
841	6	3	961	2	9	1081		1
851	8	2	971	0	6			
							100	98

	Female	Male	Difference	X
Mean and P E (mms)	858 5 ± 3 26	941 2 ± 3 40	− 82 7 ± 4 7	
S D and P E (mms)	48 3 ± 2 3	49 9 ± 2 40	− 1 6 ± 3 48	99 1
C V and P E (per cent)	5 6 ± 0 27	5 3 ± 0 26	+ 0 3 ± 0 37	

Table LXXIII

Height, Vertebral

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
1251—60 9	1		1381	7	1	1511	0	2
1261	3		1391	2	0	1521	0	3
1271	0		1401	8	0	1531	1	5
1281	3		1411	5	2	1541		3
1291	4		1421	8	0	1551		0
1301	4		1431	2	0	1561		0
1311	3		1441	5	2	1571		2
1321	6		1451	3	5	1581		1
1331	2		1461	5	4	1591		1
1341	7		1471	1	3	1601		0
1351	5		1481	2	5	1611		1
1361	7		1491	1	2	1621		0
1371	5		1501	0	2	1631		1
							100	50

	Female	Male	Difference	X
Mean and P E (mms)	1375 8 ± 4 2	1510 7 ± 5 51	− 134 9 ± 6 94	
S D and P E (mms)	62 3 ± 4 41	57 8 ± 3 90	+ 4 5 ± 5 89	90 9
C V and P E (per cent)	4 5 ± 0 22	3 8 ± 0 26	+ 0 7 ± 0 34	

Table LXXIV

Height, Lumbale

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
801—10 9	4		911	6	4	1021		5
811	1		921	10	1	1031		6
821	2		931	6	5	1041		1
	3		941	6	9	1051		1
	6		951	2	8	1061		2
	6		961	1	6	1071		0
861	11	1	971	2	5	1081		1
871	5	0	981	0	6	1091		1
881	9	2	991	1	8			
891	10	4	1001	1	5			
901	5	2	1011		4		100	90

	Female	Male	Difference	X
Mean and P E (mms)	890 6 ± 2 99	973 5 ± 3 48	− 82 9 ± 4 59	91 5
S D and P E (mms)	44 3 ± 2 11	48 9 ± 2 46	− 4 6 ± 3 24	
C V and P E (per cent)	5 0 ± 0 24	5 ± 0 25	− 0 3 ± 0 35	

Table LXXV

Height, Acromian

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
1121—30 9	2		1271	5	1	1421		3
1131	1		1281	4	4	1431		3
1141	5		1291	1	5	1441		0
1151	6		1301	4	5	1451		2
1161	4		1311	3	7	1461		0
1171	2		1321	3	11	1471		2
1181	4		1331	1	6	1481		1
1191	4		1341	0	4	1491		1
1201	7		1351	0	6	1501		0
1211	8	1	1361	0	9	1511		0
1221	4	0	1371	0	6	1521		0
1231	10	1	1381	1	5	1531		0
1241	4	2	1391		3	1541		1
1251	10	4	1401		3			
1261	7	0	1411		4			
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	1227 8 ± 3 95	1352 1 ± 4 17	− 124 3 ± 5 75	90 6
S D and P E (mms)	58 5 ± 2 79	61 8 ± 2 98	− 3 3 ± 4 75	
C V and P E (per cent)	4 8 ± 0 23	4 6 ± 0 22	+ 0 2 ± 0 32	

Table LXXVI

Sitting height, Vertex

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
701—10 9	1		801	10	4	901		2
711	1		811	13	3	911		3
721	1		821	8	8	921		0
731	2		831	3	9	931		1
741	6		841	2	4	941		0
751	10		851	2	14	951		1
761	10	2	861	1	14			
771	11	2	871	1	22			
781	9	0	881	1	5			
791	7	1	891	1	5		100	100

	Female	Male	Difference	X
Mean and P E (mms)	791 6 ± 2 43	857 6 ± 2 26	− 66 ± 3 29	
S D and P E (mms)	36 0 ± 1 72	33 4 ± 1 59	+ 2 5 ± 2 34	92 2
C V and P E (per cent)	4 6 ± 0 22	3 9 ± 0 19	+ 0 7 ± 0 29	

Table LXXVII

$$\frac{\text{Sitting height} \times 100}{\text{Total stature}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
47 5—49 4	4	6	53 5	20	10	59 5		1
49 5	27	37	55 5	1	1			
51 5	48	45	57 5		0			
							100	100

	Female	Male	Difference	X
Mean and P E	52 2 ± 0 10	51 8 ± 0 11	+ 0 3 ± 0 15	
S D and P E	1 5 ± 0 07	1 7 ± 0 08	− 0 2 ± 0 11	100 8
C V and P E	2 9 ± 0 14	3 3 ± 0 16	− 0 4 ± 0 21	

TABLE LXXVIII

$$\frac{\text{Leg length} \times 100}{\text{Trunk length}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
78 5—80 4	2	1	90 5	16	12	102 5	2	4
80 5	3	1	92 5	15	16	104 5	2	0
82 5	3	3	94 5	8	12	106 5	0	2
84 5	12	2	96 5	9	13	108 5	1	
86 5	9	7	98 5	4	5			
88 5	11	15	100 5	3	7		100	100

	Female	Male	Difference	X
Mean and P E	91 9 ± 0 40	93 8 ± 0 37	− 1 9 ± 0 54	
S D and P E	5 9 ± 0 28	5 4 ± 0 26	+ 0 4 ± 0 38	97 8
C V and P E	6 4 ± 0 31	5 8 ± 0 28	+ 0 6 ± 0 41	

TABLE LXXIX

Sitting height, Tragus

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
591—00 9	1		681	10	3	771	1	1
601	1		691	11	2	781		3
611	6		701	6	10	791		1
621	4		711	4	9	801		0
631	8	1	721	3	12	811		0
641	10	2	731	2	17	821		1
651	16	1	741	0	20			
661	7	1	751	1	5			
671	8	4	761	0	7		100	100

	Female	Male	Difference	X
Mean and P E (mms)	668 6 ± 2 35	729 0 ± 2 17	− 60 4 ± 3 2	
S D and P E (mms)	34 8 ± 1 66	32 2 ± 1 54	+ 2 6 ± 2 26	91 6
C V and P E (per cent)	5 2 ± 0 25	4 4 ± 0 21	+ 0 8 ± 0 33	

Table LXXX

Sitting height, Suprasternal

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
431—40 9	1		501	12	2	571	0	7
441	0		511	11	4	581	3	5
451	2		521	11	9	591		2
461	9		531	6	10	601		0
471	8		541	5	14	611		1
481	11	2	551	3	18	621		1
491	17	2	561	1	23			
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	525 5 ± 2	551 5 ± 1 67	— 26 ± 2 61	95 2
S D and P E (mms)	29 7 ± 1 42	24 8 ± 1 18	+ 4 9 ± 1 81	
C V and P E (per cent)	5 7 ± 2 70	4 5 ± 0 22	+ 1 2 ± 0 35	

Table LXXXI

Sitting height, Vertebral

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
571—80 9	1		661	15	2	751		0
581	2		671	9	0	761		1
591	3		681	3	8	771		0
601	8		691	3	4	781		0
611	8		701	4	9	791		0
621	12		711	3	8	801		1
631	7	1	721	0	2			
641	10	3	731	1	5			
651	10	1	741	1	0		100	45

	Female	Male	Difference	X
Mean and P E (mms)	649 5 ± 2 31	706 9 ± 3 18	— 57 4 ± 3 96	91 9
S D and P E (mms)	34 3 ± 1 64	33 3 ± 2 25	+ 1 0 ± 2 78	
C V and P E (per cent)	5 3 ± 0 25	4 8 ± 0 33	+ 0 5 + 4 09	

Table LXXXII

Sitting height, Iliо-cristal

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
151—55 9	1		186	14	7	221	3	9
156	0		191	15	6	226	1	4
161	3		196	10	9	231	1	2
166	2		201	14	17	236		1
171	1		206	14	12	241		0
176	4	1	211	3	9	246		1
181	13	5	216	1	12			
							100	95

	Female	Male	Difference	X
Mean and P E (mms)	194 4 ± 0 96	206 9 ± 0 96	— 12 5 ± 1 1	94 4
S D and P E (mms)	14 2 ± 0 68	13 9 ± 0 68	+ 0 3 ± 1 0	
C V and P E (per cent)	7 3 ± 0 35	6 7 ± 0 33	+ 0 6 ± 0 48	

Table LXXXIII

Bracromial diameter

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
286—90 9	2		336	15	3	386	1	5
291	3		341	4	3	391		8
296	1		346	5	4	396		4
301	4		351	4	5	401		5
306	9	1	356	1	6	406		1
311	10	0	361	0	13	411		1
316	10	1	366	1	13	416		1
321	9	0	371	0	9			
326	16	1	376	0	6			
331	5	1	381	0	9		100	100

	Female	Male	Difference	X
Mean and P E (mms)	327 3 ± 0 81	372 9 ± 1 31	— 45 7 ± 1 54	87 7
S D and P E (mms)	11 9 ± 0 57	19 4 ± 0 93	— 7 5 ± 1 09	
C V and P E (per cent)	3 7 ± 0 17	5 2 ± 0 25	— 1 6 ± 0 34	

Table LXXXIV

Ilio-cristal diameter

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
216—220	1	1	251	8	12	286	3	1
221	0	4	256	10	10	291	1	1
226	0	3	261	19	8	296	1	1
231	5	7	266	9	9	301	1	
236	3	12	271	10	6			
241	6	6	276	7	2			
246	13	13	281	3	2		100	98

	Female	Male	Difference	X
Mean and P E (mms)	260 3 ± 1 04	251 8 ± 1 13	+ 8 5 ± 1 53	
S D and P E (mms)	15 4 ± 0 73	16 6 ± 0 80	— 1 2 ± 1 08	103 5
C V and P E (per cent)	5 9 ± 0 28	6 6 ± 0 32	— 0 7 ± 0 43	

Table LXXXV

$$\frac{\textit{Ilio cristal diameter} \times 100}{\textit{Biacromical diameter}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
57 5—59 4		3	71 5	4	7	85 5	0	
59 5		6	73 5	4	7	87 5	3	
61 5		4	75 5	17	2	89 5	1	
63 5		12	77 5	19	1	91 5	2	
65 5		21	79 5	21	1			
67 5		22	81 5	10				
69 5	1	12	83 5	9			100	98

	Female	Male	Difference	X
Mean and P E	80 5 ± 0 30	67 9 ± 0 29	+ 12 6 ± 0 42	
S D and P E	4 4 ± 0 21	4 3 ± 0 21	+ 0 1 ± 0 30	118 6
C V and P E	5 4 ± 0 26	6 4 ± 0 31	— 0 9 ± 0 40	

TABLE XC

Binammillary diameter (males only)

Class mms	Z Male	Class mms	Z Male	Class mms	Z Male	Class mms	Z Male
1521—25 9	2	1541	18	1561	8	1581	3
1526	2	1546	15	1566	9		
1531	4	1551	13	1571	6		
1536	3	1556	12	1576	5		100

Mean and P E (mms)	183 7 ± 0 92
S D and P E (mms)	13 7 ± 0 65
C V and P E (per cent)	7 4 ± 0 36

TABLE XCI

$$\frac{\textit{Binammillary diameter} \times 100}{\textit{Biacromial diameter}}$$ *(males only)*

Class per cent	Z Male	Class per cent	Z Male	Class per cent	Z Male	Class per cent	Z Male
40 5—42 4	3	46 5	14	52 5	8	58 5	3
42 5	7	48 5	23	54 5	8		
44 5	13	50 5	17	56 5	4		100

Mean and P E	49 8 ± 0 29
S D and P E	4 3 ± 0 21
C V and P E	8 7 ± 0 53

TABLE XCII

Ilio-spinal diameter

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
171—75 9	1		211	6	11	251	2	2
176	1		216	11	17	256	2	1
181	1		221	10	11	261	1	
186	2		226	11	12	266	1	
191	2	3	231	11	10	271	0	
196	5	5	236	10	8	276	1	
201	8	4	241	6	2			
206	4	8	246	4	4		100	98

	Female	Male	Difference	X
Mean and P E (mms)	222 9 ± 1 31	221 5 ± 0 98	+ 1 4 ± 1 63	
S D and P E (mms)	19 4 ± 0 92	14 4 ± 0 69	+ 5 0 ± 1 15	100 9
C V and P E (per cent)	8 5 ± 0 51	6 5 ± 0 55	+ 1 2 ± 0 75	

TABLE XCIII

$$\frac{\textit{Ilio spinal diameter} \times 100}{\textit{Ilio cristal diameter}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
70 5—72 4	4	1	82 5	13	11	94 5	3	6
72 5	0	0	84 5	15	12	96 5	3	5
74 5	2	0	86 5	16	17	98 5		2
76 5	2	4	88 5	12	9	100 5		1
78 5	7	3	90 5	8	17			
80 5	10	5	92 5	5	5		100	98

	Female	Male	Difference	X
Mean and P E	85 8 ± 0 38	88 1 ± 0 41	— 2 2 ± 0 55	
S D and P E	5 6 ± 0 27	5 9 ± 0 29	— 0 3 ± 0 39	97 4
C V and P E	6 5 ± 0 31	6 7 ± 0 32	— 0 2 ± 0 45	

Table XCIV

Dorso-ventral diameter of thorax plane I (males only)

Class mms	Z Male	Class mms	Z Male	Class mms	Z Male	Class mms	Z Male
135 5—40 4	1	155 5	5	175 5	3	195 5	1
140 5	2	160 5	7	180 5	5		
145 5	3	165 5	3	185 5	3		
150 5	7	170 5	9	190 5	2		51

Mean and P E (mms)	166 8 ± 1 37
S D and P E (mms)	14 4 ± 0 97
C V and P E (per cent)	8 6 ± 0 58

Table XCV

Transverse diameter of thorax plane I

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
196—00 9	1		231	12	5	266	1	11
201	4		236	1	5	271	1	7
206	9		241	4	9	276		2
211	14	1	246	2	7	281		2
216	16	0	251	1	11	286		1
221	16	1	256	0	14			
226	18	4	261	0	17		100	97

	Female	Male	Difference	X
Mean and P E (mms)	222 7 ± 0 85	257 8 ± 1 13	— 35 1 ± 1 42	
S D and P E (mms)	12 6 ± 0 60	16 6 ± 0 80	— 3 9 ± 1 00	86 4
C V and P E (per cent)	5 7 ± 0 27	6 4 ± 0 31	— 0 7 ± 0 42	

TABLE XCVI

Dorso-ventral diameter of thorax plane II (males only)

Class mms	Z Male	Class mms	Z Male	Class mms	Z Male	Class mms	Z Male
140 5—45 4	2	160 5	11	180 5	7	200 5	1
145 5	5	165 5	9	185 5	0		
150 5	2	170 5	5	190 5	2		
155 5	6	175 5	1	195 5	0		51

Mean and P E (mms)	166 3 ± 1 21
S D and P E (mms)	12 8 ± 0 86
C V and P E (per cent)	8 4 ± 0 57

TABLE XCVII

Transverse diameter of thorax plane II

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
201—05 9	2		241	12	6	281		7
206	5		246	9	8	286		0
211	7	1	251	6	9	291		3
216	8	0	256	1	8	296		1
221	11	2	261	0	9	301		2
226	11	0	266	3	18	306		0
231	13	2	271	0	13	311		1
236	11	2	276	1	5		100	97

	Female	Male	Difference	X
Mean and P E (mms)	232 5 ± 1 02	263 4 ± 1 15	— 30 9 ± 1 53	
S D and P E (mms)	15 1 ± 0 72	16 8 ± 0 81	— 1 6 ± 1 09	88 4
C. V and P E (per cent)	6 5 ± 0 31	6 4 ± 0 31	+ 0 2 ± 0 44	

TABLE XCVIII

$$\frac{\text{Thorax plane II dorso ventral diameter} \times 100}{\text{Thorax plane II transverse diameter}}$$ (*males only*)

Class per cent	Z Male	Class per cent	Z Male	Class per cent	Z Male	Class per cent	Z Male
51 5—53 4	1	59 5	6	67 5	5	75 5	1
53 5	1	61 5	8	69 5	1		
55 5	2	63 5	11	71 5	0		
57 5	4	65 5	6	73 5	3		50
Mean and P E					63 6 ± 0 47		
S D and P E					4 9 ± 0 33		
C V and P E					7 7 ± 0 52		

TABLE XCIX

Girth of neck across larynx

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
261—65 9	3		301	7		341	1	10
266	3		306	5	4	346		9
271	5		311	2	7	351		6
276	17		316	2	4	356		4
281	12		321	2	15	361		4
286	16		326	1	8	366		1
291	16		331	2	11			
296	6		336	0	15		100	98

	Female	Male	Difference	X
Mean and P E (mms)	290 2 ± 1 05	334 5 ± 0 99	− 44 3 ± 1 44	86 9
S D and P E (mms)	15 5 ± 0 74	14 5 ± 0 70	+ 1 0 ± 1 02	
C V and P E (per cent)	5 3 ± 0 26	4 4 ± 0 21	+ 0 9 ± 0 33	

(*b*) SUMMARY AND DISCUSSIONS OF THE TRUNK MEASUREMENTS

Measurements of the male group

Average stature was found to be 1660 mm (Table LVI) which compares with stature of Bene-israel from the report Weight of the body is 52 3 kg (Table LVII) Each centimetre height is equal to 318 3 grammes (Table LVIII) Constitutional index is 22 6 (Table LIX), only 15 individuals being above the

average which indicates a fair constitution Maximum girth of thorax is 805 5 mm (Table LX) Table LXI of a constitutional index indicates that 3 persons have very strong constitution (0–15) Seven individuals have very good constitution (15–20) Thirteen have very fair constitution (20–25) Twelve have weak constitutions (25–30) Twenty have very weak constitution (30–35), and the rest 42 individuals have very bad constitution (35 onwards) An average constitution 32–45 being very weak Girth of thorax at Xiphoid process is 760 5 mm (Table LXII), and girth at waist is 662 5 mm (Table LXIII) Height to tragus is 1535 6 mm (Table LXIV) and height to the chin is 1455 9 mm (Table LXV) which is the same as that of Dakshini Vanjari from the report Suprasternal height is 1360 9 mm (Table LXVI), which compares with that of Namdev Simpi Height to the nipple is 1235 5 mm (Table LXVII) and mesosternal height is 1265 4 mm (Table LXVIII) Umbilical height is 1014 5 mm (Table LXIX) and height to the symphysis pubis is 862 mm (Table LXX) Ilio-cristal height is 1009 6 mm (Table LXXI), while ilio-spinal height is 941 2 mm (Table LXXII) Height of the level of 2nd cervical vertebra is 1510 7 mm (Table LXXIII) and height of the 5th lumbar spine is 973 5 mm (Table LXXIV) Acromial height is 1352 mm (Table LXXV) Sitting height Vertex is 857 6 mm (Table LXXVI) which is nearly the same as that of Bene-israel from the report Proportion of the trunk to the total stature is 51 8 per cent, while the leg to trunk proportion is 93 8 per cent (Table LXXVIII) indicating the average to be sub-makro-skelic (90–95) Sitting height Tragus is 729 mm (Table LXXIX) Sitting height Suprasternal is 551 5 mm (Table LXXX) The length of the whole Vertebral column as indicated by the sitting height Vertebral is 706 9 mm (Table LXXXI) Sitting height Ilio-cristal indicating the Pelvic height is 206 85 mm (Table LXXXII) Breadth of trunk between the acromial points is 372 9 mm (Table LXXXIII) Greatest pelvic transverse diameter indicated by Ilio-cristal diameter is 251 8 mm (Table LXXXIV) and is nearly the same as for Kolis and Namdev Simpis from the report Proportion of pelvic breadth to the acromial diameter is 67 9 per cent (Table LXXXV) Distance between the outer surface of greater trochanters is 297 7 mm (Table LXXXVI) The proportion of bitrochanteric diameter to biacromial diameter is 80 33 per cent (Table LXXXVII) Distance between the outer surfaces of deltoids is 413 6 mm (Table LXXXVIII), while the pelvic breadth to shoulders is 61 per cent (Table LXXXIX) The distance between the nipples is 183 7 mm (Table XC) and the bimammillary diameter to biacromial diameter is 49 8 per cent (Table XCI) Distance between the anterior superior Ilio-spines is 221 5 mm (Table XCII) while Ilio-spinal to Ilio-cristal proportion is 88 08 per cent (Table XCIII) Thoracic depth at Xiphoid process is 166 75 mm (Table XCIV) and breadth of chest at Xiphoid process is 257 75 mm (Table XCV) Depth of thorax at mesosternal point is 166 25 mm (Table XCVI) Breadth at the same point is 263 35 mm (Table XCVII), relation of depth to breadth of thorax at mesosternal

point is 63 62 per cent (Table XCVIII) and girth of neck is 334 5 mm (Table XCIX)

The least variation 3 8 per cent is about the Vertebral height (Table LXXIII) and the greatest 11 68 per cent about the weight of the body (Table LVII)

In indices, the subjects deviate least about the mean of Sitting height total stature index and the variation is 3 26 per cent (Table LXXVII), while the greatest variation has been found in constitutional index (Table LIX) being 29 95 per cent

Measurements of the female group

The average total stature of the female group is 1518 4 mm (Table LVI) The different studies in America give a standard total stature for American women ranging from 1596 5 mm to 1638 mm The weight is 45 8 kg (Table LVII) The figures for America range from 52 45 to 56 62 kg Each centimetre of height is equal to 304 2 grammes (Table LVIII) The constitutional index is 23 6 (Table LIX) Tragion height is 1405 6 mm (Table LXIV), while the Americans have 1500 45 to 1510 mm Chin height is 1312 8 mms (Table LXV) Supra-sternal height is 1235 8 mm (Table LXVI) Ilio-cristal height is 917 5 mm (Table LXXI), while American women have 955 65 mm Ilio-spinal height is 858 5 mm (Table LXXII) Americans' height ranges from 903 8 to 926 7 mm Vertebral height is 1375 8 mm (Table LXXIII) Lumbar height is 890 6 mm (Table LXXIV) Height acromial is 1227 8 mm (Table LXXV) Americans have 1314 to 1324 mm Sitting height is 791 6 mm (Table LXXVI) Americans' Sitting height is 868 4 mm Relation of Sitting height to total height is 52 2 per cent (Table LXXVII), while the Americans' relative Sitting height is 53 28 per cent The length of legs is in relation of 91 9 per cent to the Trunk length (Table LXXVIII) and the body is sub-makro-skelic Sitting height tragus is 668 6 mm (Table LXXIX) Sitting height suprasternal is 525 5 mm (Table LXXX) Sitting height vertebral is 649 5 mm (Table LXXXI) indicating the height of the vertebral column Sitting height Ilio-cristal indicating Pelvic height is 194 4 mm (Table LXXXII) Biacromial diameter is 327 25 mm (Table LXXXIII), while Americans have from 351 to 356 mm Ilio-cristal diameter is 260 25 mm (Table LXXXIV), while Americans have a range from 263 to 272 mm Trunk index showing proportion of Ilio-cristal breadth to Biacromial breadth is 80 5 per cent (Table LXXXV) The trunk index of Americans is 77–79 49 This shows the greater relative breadth of pelvis of Indian women Bitrochanteric diameter is 295 mm (Table LXXXVI), and of Americans' figures, one 236 mm does not seem to be accurate, while a figure from the other series 318 5 mm seems to be the mean for American women Relation of bitrochanteric diameter to biacromial diameter is 91 02 per cent (Table LXXXVII) Breadth of shoulders between deltoids is 365 5 mm (Table LXXXVIII) Ilio-cristal diameter is 71 5 per cent

of the breadth of shoulders (Table LXXXIX) Ilio-spinal diameter is 222 85 mm (Table XCII), while Americans have 244 35 mm Ilio-spinal diameter is 85 8 per cent of the Ilio-cristal diameter (Table XCIII) Transverse diameter of chest at Xiphoid process is 222 7 mm (Table XCV), and at mesosternal plane is 232 5 mm (Table XCVII) The girth of neck is 290 mm (Table XCIX)

The least proportion of deviation 3 65 per cent to the mean is borne by biacromial diameter (Table LXXXIII) The greatest variation 15 6 per cent is in weights (Table LVII)

In indices the least variation 2 87 per cent is in trunk-height index (Table LXXVII) The greatest variation being in constitutional index (Table LVIII) 11 85 per cent

Comparison of male and female standards (Fig 2)

Transverse diameter of thorax at Xiphoid process, and girth of neck of female group are 86 per cent of the male group (Tables XCV and XCIX) Weights and biacromial diameter are 87 per cent of the male (Tables LVII and LXXXIII) Breadth of shoulders between deltoids and transverse diameter of thorax at mesosternal plane are 88 4 per cent (Tables LXXXVIII and XCVII) Chin height, Suprasternal height, Ilio-cristal height, Vertebral and Acromial heights are 90 per cent (Tables LXV, LXVI, LXXI, LXXIII and LXXV) Total stature, Tragion height, Ilio-spinal Lumbar height, and Sitting tragus and Vertebral heights are 91 per cent (Tables LVI, LXIV, LXXII, LXXIV, LXXIX and LXXXI) Sitting vertex is 92 per cent (Table LXXVI) Sitting Ilio-cristal height is 94 per cent (Table LXXXII) Sitting suprasternal height is 95 per cent (Table LXXX) Bitrochanteric distance is 99 per cent (Table LXXXVI) Ilio-spinal diameter is practically the same as male 100 8 per cent (Table XCII) Ilio-cristal diameter is above the male average 103 5 per cent (Table LXXXIV)

Proportion of weights to the stature of the female group is 95 per cent of the male group (Table LVIII) The proportion of leg to Trunk and Ilio-spinal diameter to Ilio-cristal diameter are 97 per cent of the male ones (Tables LXXVII and XCIII) The Trunk to total stature is 100 8 per cent of the male one (Table LXXVII) The constitutional index is 104 4 per cent of the male one (Table LIX) Relation of bitrochanteric diameter to biacromial diameter is 113 3 per cent of the male (Table LXXXVII) The proportion of Ilio-cristal diameter to Biacromial diameter is 118 6 per cent of the male one (Table LXXXV) Ilio-cristal diameter to breadth of shoulders is 117 2 of the male proportion (Table LXXXIX)

Aggregate average trunk of the female is 91 85 per cent of the male body, including the pelvic measurements

Aggregate average of indices of female trunk is 104 4 per cent of the male trunk indices,

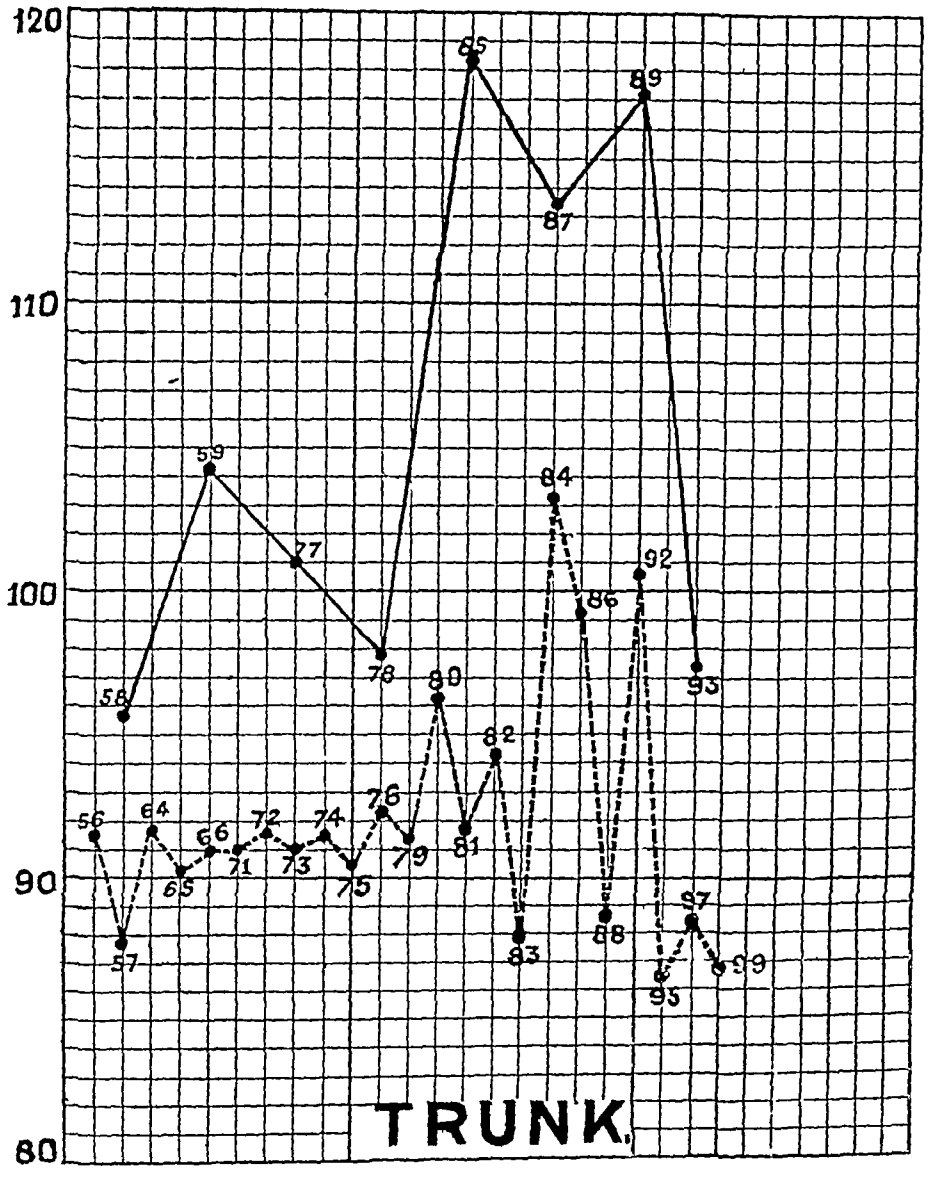

FIG 2

Comparison of trunk study of males and females

A graph showing the comparison of the standards of trunk measurements and indices The standards of the female measurements and indices have been reduced to the percentage of the male standards Thus all the male standards represent the line 100 The numbered dots along the broken line indicate the table numbers of measurements of female group, and those along the unbroken line indicate indices of the same The numbers refer to table numbers

Dot numbers along the broken line (measurements)

56 Tot Stature
57 Weights
64 Tragion ht.
65 Gnathion „
66 Suprast „
71 Ilio cristal „
72 Ilio spinal ht
73 Vertebral „
74 Lumbale „
75 Acromian „
76 St ht. vertex
79 „ „ tragus
80 St ht suprast
81 „ „ vertebra
82 „ „ ilio crist
83 Biacromial D
84 Ilio crist „
86 Bitroch „
88 Breadth of shoulders
92 Ilio spin diam
95 Trans D of thorax I
97 „ „ „ „ II
99 Neck girth across larynx

Dot numbers along the unbroken line (indices)

58 $\frac{\text{Wt in gs}}{\text{Stat in cms}}$

59 $\frac{\sqrt[3]{\text{Weight}}}{\text{Stature}}$

77 $\frac{\text{Sitting ht}}{\text{Tot stature}}$

78 $\frac{\text{Leg length}}{\text{Trunk length}}$

85 $\frac{\text{Ilio cris D}}{\text{Ilio spinal D}}$

87 $\frac{\text{Bitroch D}}{\text{Biacromial D}}$

89 $\frac{\text{Ilio cristal diam}}{\text{Breadth of shoulder}}$

93 $\frac{\text{Ilio spinal diam}}{\text{Ilio cristal D}}$

Study of Appendages

Supplementary notes to 'Methods for taking measurements and observations' (p 164)

Length of entire arm with hand=ht Acromion *minus* ht Dactylion
Length of entire arm without hand=ht Acromion *minus* ht Stylion
Length of upper arm=ht Acromion *minus* ht Radial
Length of forearm=ht Radial *minus* ht Stylion
Total leg length is the mean of height symphysion and height Ilio-cristal in case of males
In females we have taken the leg length as Ilio-spinal height *minus* 40 mm
Total leg length *minus* ht Spherion is leg length without foot
Length of thigh is total leg *minus* ht Tibial
Length of lower leg is ht Tibial *minus* ht Spherion

(*a*) Tables of measurements and indices of appendages

Table C

Arm stretch

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
1391—00 9	1		1581	3	2	1771		3
1401	1		1591	7	2	1781		2
1411	0		1601	5	2	1791		3
1421	0		1611	5	1	1801		3
1431	1		1621	4	3	1811		3
1441	3		1631	2	2	1821		4
1451	5		1641	6	4	1831		2
1461	0		1651	1	7	1841		2
1471	2		1661	0	2	1851		5
1481	4		1671	0	2	1861		1
1491	4		1681	1	2	1871		1
1501	7		1691		5	1881		2
1511	6		1701		4	1891		0
1521	5		1711		10	1901		0
1531	5		1721		6	1911		0
1541	9		1731		2	1921		1
1551	4		1741		4			
1561	5		1751		4			
1571	4		1761		4		100	100

	Female	Male	Difference	X
Mean and P E (mms)	1553 4 ± 4 0	1734 3 ± 5 34	— 180 9 ± 6 68	
S D and P E (mms)	59 8 ± 2 9	79 2 ± 3 78	— 19 4 ± 4 73	89 6
C V and P E (per cent)	3 9 ± 0 2	4 6 ± 0 22	— 0 7 ± 0 29	

Table CI

$$\frac{\textit{Arm stretch} \times 100}{\textit{Total stature}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
95 5—97 4	2		101 5	32	25	107 5	1	6
97 5	12	2	103 5	19	31	109 5		2
99 5	25	9	105 5	9	25			
							100	100

	Female	Male	Difference	X
Mean and P E	102 2 ± 0 16	104 4 ± 0 16	− 2 2 ± 0 23	98
S D and P E	2 4 ± 0 12	2 4 ± 0 12	− 0 0 ± 0 16	
C V and P E	2 4 ± 0 11	2 3 ± 0 11	+ 0 1 ± 0 16	

Table CII

Height, Radial

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
861—70 9	3		971	7	1	1081		3
871	5		981	5	6	1091		3
881	5		991	3	7	1101		4
891	6		1001	6	6	1111		3
901	5		1011	4	16	1121		0
911	9		1021	1	4	1131		3
921	3	2	1031	0	7	1141		0
931	12	0	1041	0	10	1151		0
941	4	2	1051	0	12	1161		0
951	12	2	1061	0	4	1171		1
961	9	2	1071	1	2			
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	944 2 ± 2 89	1040 ± 3 19	− 95 8 ± 4 32	91 1
S D and P E (mms)	42 8 ± 2 04	47 3 ± 2 26	− 4 5 ± 3 04	
C V and P E (per cent)	4 5 ± 0 22	4 6 ± 0 22	− 0 1 ± 0 31	

Table CIII

Girth of upper arm, greatest

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
201—05 9	2		251	7	12	301	1	1
206	5		256	6	8	306	0	5
211	2		261	2	10	311	0	1
216	6		266	8	8	316	0	1
221	4		271	2	8	321	1	0
226	8		276	3	8	326	1	0
231	9		281	1	5	331		1
236	8	4	286	2	9	336		0
241	12	4	291	1	4	341		1
246	7	7	296	2	2			
							100	99

	Female	Male	Difference	X
Mean and P E (mms)	245 2 ± 1 99	270 9 ± 1 44	− 25 7 ± 2 46	90 5
S D and P E (mms)	29 6 ± 1 41	21 3 ± 1 02	+ 8 3 ± 1 74	
C V and P E (per cent)	12 1 ± 0 58	7 9 ± 0 38	+ 4 2 ± 0 22	

Table CIV

$$\frac{\textit{Girth of upper arm} \times 100}{\textit{Length of upper arm}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
65—69 9	1	1	90	12	13	115	1	
70	10	9	95	10	2	120	1	
75	15	11	100	5	5			
80	20	22	105	3	1			
85	21	35	110	1			100	99

	Female	Male	Difference	X
Mean and P E	87 2 ± 0 71	85 4 ± 0 51	+ 1 8 ± 0 87	102 1
S D and P E	10 5 ± 0 50	7 7 ± 0 36	+ 2 8 ± 0 62	
C V and P E	12 1 ± 0 58	8 9 ± 0 43	+ 3 2 ± 0 72	

TABLE CV

Girth across contracted biceps

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
196—00 9	1		256	5	9	316	1	0
201	0		261	6	7	321	0	2
206	6		266	6	8	326	0	
211	3		271	2	9	331	0	
216	9		276	2	7	336	0	
221	3		281	1	12	341	1	
226	2	2	286	3	5	346	0	
231	11	3	291	0	2	351	0	
236	11	5	296	0	2	356	1	
241	6	5	301	0	2			
246	6	6	306	0	1			
251	10	8	311	1	4		100	99

	Female	Male	Difference	X
Mean and P E (mms)	246 9 ± 1 88	268 3 ± 1 47	− 21 5 ± 2 38	91 9
S D and P E (mms)	27 9 ± 1 33	21 7 ± 1 04	+ 6 2 ± 1 67	
C V and P E (per cent)	11 3 ± 0 55	8 1 ± 0 39	+ 3 2 ± 0 67	

TABLE CVI

Height, Stylion

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
641—50 9	2		741	8	6	841	1	5
651	2		751	8	9	851		3
661	2		761	4	7	861		1
671	4		771	4	14	871		0
681	12		781	2	14	881		0
691	4	1	791	3	12	891		1
701	9	3	801	1	9			
711	10	2	811	1	3			
721	11	2	821	0	1			
731	12	3	831	0	4		100	100

	Female	Male	Difference	X
Mean and P E (mms)	725 5 ± 2 74	783 5 ± 2 57	− 58 ± 3 76	92 7
S D and P E (mms)	40 6 ± 2 44	38 2 ± 1 82	+ 2 5 ± 3 05	
C V and P E (per cent)	5 6 ± 0 27	4 9 ± 0 23	+ 0 6 ± 0 36	

Table CVII

$$\frac{\text{Lower arm} \times 100}{\text{Upper arm}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
50 5—54 9	1		75	38	54	100		1
55	0		80	24	22	105		0
60	1	2	85	9	6	110		1
65	5	2	90		0			
70	22	11	95		1		100	100

	Female	Male	Difference	X
Mean and P E	77 6 ± 0 38	76 9 ± 0 42	+ 0 7 ± 0 57	
S D and P E	5 7 ± 0 27	6 3 ± 0 30	− 0 6 ± 0 40	100 9
C V and P E	7 3 ± 0 35	8 1 ± 0 39	− 0 9 ± 0 52	

Table CVIII

Girth of forearm, greatest

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
186—90 9	3		221	8	7	256	0	8
191	3		226	9	10	261	0	2
196	6		231	7	10	266	1	3
201	10		236	2	19	271	1	
206	26		241	3	14			
211	8		246	1	13		100	98
216	12	6	251	0	6			

	Female	Male	Difference	X
Mean and P E (mms)	215 ± 1 04	239 7 ± 0 89	− 24 7 ± 1 36	
S D and P E (mms)	15 4 ± 0 73	12 9 ± 0 62	+ 2 4 ± 0 96	89 8
C V and P E (per cent)	7 2 ± 0 34	5 4 ± 0 26	+ 1 7 ± 0 43	

TABLE CIX

$$\frac{\text{Girth of forearm} \times 100}{\text{Length of forearm}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
80—81 9	3	7	105	17	10	130	0	
85	14	13	110	4	2	135	1	
90	24	23	115	4	1			
95	21	21	120	1			100	98
100	11	21	125	0				

	Female	Male	Difference	X
Mean and P E	98 8 ± 0 64	96 5 ± 0 52	+ 2 2 ± 0 68	
S D and P E	9 5 ± 0 45	7 5 ± 0 37	+ 2 0 ± 0 58	104 6
C V and P E	9 6 ± 0 46	7 8 ± 0 38	+ 1 8 ± 0 60	

TABLE CX

$$\frac{\text{Girth of forearm} \times 100}{\text{Girth of upper arm}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
75—79 9	6	1	95	6	1	115		1
80	27	16	100	2	0			
85	32	44	105		1		100	98
90	27	34	110		0			

	Female	Male	Difference	X
Mean and P E	87 8 ± 0 36	88 9 ± 0 34	— 1 1 ± 0 49	
S D and P E	5 3 ± 0 25	4 9 ± 0 24	+ 0 4 ± 0 35	98 7
C V and P E	6 0 ± 0 29	5 5 ± 0 24	+ 0 5 ± 0 36	

Table CXI

Height, Dactylion

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
471—80 9	1		551	12	6	631	1	4
481	1		561	8	7	641	0	6
491	4		571	10	8	651	0	3
501	6		581	7	12	661	1	2
511	12		591	2	13	671		0
521	10	2	601	4	14	681		0
531	5	4	611	3	8	691		1
541	12	2	621	1	8			
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	550 7 ± 2 41	597 6 ± 2 25	— 46 9 ± 3 3	92
S D and P E (mms)	35 7 ± 1 70	33 4 ± 1 59	+ 2 3 ± 2 34	
C V and P E (per cent)	6 5 ± 0 31	5 6 ± 0 27	+ 0 9 ± 0 41	

Table CXII

$$\frac{\textit{Acromian minus stylion} \times 100}{\textit{Total stature}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
28—28 9	1		33	29	27	38		0
29	0	1	34	25	27	39		0
30	2	0	35	5	19	40		1
31	13	4	36		7			
32	25	14	37		0			
							100	100

	Female	Male	Difference	X
Mean and P E	33 3 ± 0 09	34 2 ± 0 11	— 0 9 ± 0 15	97 4
S D and P E	1 4 ± 0 07	1 6 ± 0 08	— 0 2 ± 0 10	
C V and P E	4 3 ± 0 21	4 7 ± 0 22	— 0 4 ± 0 31	

TABLE CXIII

$$\frac{\text{Upper arm} \times 100}{\text{Total arm with hand}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
36—36 9		1	41	26	33	46	0	
37	1	0	42	33	30	47	0	
38	1	0	43	9	13	48	1	
39	1	2	44	2	5	49	1	
40	21	12	45	1	4			
							100	100

	Female	Male	Difference	X
Mean and P E	41 9 ± 0 10	42 2 ± 0 91	— 0 3 ± 0 92	99 4
S D and P E	1 6 ± 0 08	1 4 ± 0 65	+ 0 2 ± 0 69	
C V and P E	3 8 ± 0 18	3 2 ± 0 15	+ 0 6 ± 0 24	

TABLE CXIV

$$\frac{\text{Lower arm} \times 100}{\text{Total arm with hand}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
25—25 9	1		33	25	39	41		0
26	0		34	13	12	42		0
27	0		35	2	3	43		0
28	0	1	36		2	44		1
29	2	1	37		0			
30	14	1	38		1			
31	19	13	39		0			
32	24	26	40		0		100	100

	Female	Male	Difference	X
Mean and P E	32 5 ± 0 10	33 3 ± 0 12	— 0 8 ± 0 16	97 6
S D and P E	1 5 ± 0 07	1 7 ± 0 08	— 0 2 ± 0 11	
C V and P E	4 7 ± 0 22	5 2 ± 0 24	— 0 6 ± 0 33	

Table CXV

Girth of wrist, least

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
121—125 9	1		141	26	2	161	1	16
126	1		146	14	15	166		5
131	19	1	151	5	27	171		4
136	28	1	156	2	27		100	98

	Female	Male	Difference	X
Mean and P E (mms)	139 9 ± 0 49	155 8 ± 0 49	— 15 8 ± 0 70	
S D and P E (mms)	7 3 ± 0 35	7 3 ± 0 35	+ 0 0 ± 0 49	89 7
C V and P E (per cent)	5 5 ± 0 26	4 7 ± 0 22	+ 0 8 ± 0 34	

Table CXVI

$$\frac{\text{Girth of wrist} \times 100}{\text{Girth of upper arm}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
45 5—47 4	2		55 5	16	18	65 5	3	1
47 5	3	2	57 5	15	22	67 5	1	
49 5	5	2	59 5	13	19			
51 5	6	5	61 5	9	10			
53 5	19	16	63 5	8	3		100	98

	Female	Male	Difference	X
Mean and P E	57 6 ± 0 31	57 9 ± 0 24	— 0 3 ± 0 39	
S D and P E	4 6 ± 0 22	3 5 ± 0 17	+ 1 2 ± 0 28	99 5
C V and P E	8 1 ± 0 39	6 0 ± 0 29	+ 2 1 ± 0 48	

Table CXXI

Hand breadth / Hand length × 100

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
32—32 9	1		39	10	2	46	4	8
33	0		40	15	7	47		3
34	0		41	14	15	48		3
35	0		42	15	21	49		2
36	0		43	17	17	50		1
37	3		44	14	10			
38	2	2	45	5	8			
							100	99

	Female	Male	Difference	X
Mean and P E	42 1 ± 0 17	43 6 ± 0 16	− 1 5 ± 0 23	96 6
S D and P E	2 5 ± 0 12	2 4 ± 0 12	+ 0 1 ± 0 17	
C V and P E	5 9 ± 0 28	5 5 ± 0 27	+ 0 4 ± 0 39	

Table CXXII

Height, Trochanterian

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
701—10 9	3	1	811	12	5	921		4
711	1	0	821	11	4	931		6
721	2	0	831	9	3	941		3
731	4	0	841	3	6	951		3
741	2	0	851	4	10	961		4
751	4	0	861	0	11	971		1
761	10	0	871	3	5	981		0
771	8	0	881	1	10	991		0
781	10	2	891	0	5	1001		1
791	6	2	901	1	7			
801	6	2	911		5			
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	798 2 ± 2 77	880 0 ± 3 64	− 81 8 ± 4 57	90 7
S D and P E (mms)	41 1 ± 2 47	53 9 ± 2 57	− 12 8 ± 3 68	
C V and P E (per cent)	5 1 ± 0 25	6 1 ± 0 29	− 1 0 ± 0 38	

TABLE CXXIII

Height, Tibiale

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
351—355	1		406	8	5	461	0	9
356	1		411	7	0	466	1	5
361	4		416	10	11	471		3
366	4		421	5	2	476		8
371	3		426	7	9	481		2
376	4		431	2	7	486		2
381	8		436	5	5	491		0
386	5		441	2	4	496		1
391	12		446	1	7	501		0
396	6	1	451	1	6	506		1
401	2	4	456	1	8			
							100	100

	Female	Male	Difference	X
Mean and P E (mms)	402 4 ± 1 78	445 1 ± 1 66	− 42 7 ± 2 43	90 4
S D and P E (mms)	26 4 ± 1 26	24 7 ± 1 18	+ 1 8 ± 2 18	
C V and P E (per cent)	6 6 ± 0 31	5 5 ± 0 27	+ 1 0 ± 1 03	

TABLE CXXIV

$$\frac{\textit{Upper arm} \times 100}{\textit{Length of thigh}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
54—55 9	1		68	29	16	82	1	0
56	0	2	70	20	19	84		1
58	1	1	72	3	17	86		0
60	2	1	74	5	3	88		1
62	7	4	76	0	2			
64	12	10	78	2	1			
66	15	21	80	2	0		100	99

	Female	Male	Difference	X
Mean and P E	68 8 ± 0 29	69 4 ± 0 31	− 0 6 ± 0 43	99 2
S D and P E	4 3 ± 0 21	4 6 ± 0 22	− 0 3 ± 0 30	
C V and P E	6 3 ± 0 30	6 7 ± 0 32	− 0 4 ± 0 44	

TABLE CXXV

Maximum girth of thigh (males only)

Class mms	Z Male	Class mms	Z Male	Class mms	Z Male	Class mms	Z Male
390—94 9	1	440	1	490	5	540	2
395	1	445	1	495	2	545	2
400	1	450	7	500	3	550	0
405	6	455	4	505	4	555	1
410	0	460	5	510	3	560	0
415	5	465	5	515	2	565	0
420	4	470	5	520	2	570	0
425	1	475	5	525	1	575	1
430	1	480	5	530	0		
435	2	485	4	535	0		95

Mean and P E (mms)	469 5 ± 3 01
S D and P E (mms)	39 6 ± 1 94
C V and P E (per cent)	8 4 ± 0 42

TABLE CXXVI

$$\frac{\textit{Girth of thigh greatest} \times 100}{\textit{Length of thigh}} \quad \textit{(males only)}$$

Class per cent	Z Male	Class per cent	Z Male	Class per cent	Z Male	Class per cent	Z Male
80—84 9	2	95	11	110	10	125	2
85	10	100	29	115	6	130	2
90	11	105	11	120	1		95

Mean	Mean and P E	102 7 ± 0 72
S D	S D and P E	10 4 ± 0 51
C V a	C V and P E	10 1 ± 0 50

TABLE CXXVII

Girth of thigh, middle

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
356—360 9		1	461	0	6	566	1	
361		0	466	2	3	571	0	
366		0	471	2	3	576	2	
371		1	476	4	2	581	1	
376		0	481	3	3	586	3	
381		0	486	4	3	591	1	
386		3	491	5	3	596	0	
391		2	496	4	2	601	0	
396		2	501	5	2	606	1	
401		1	506	3	2	611	2	
406		1	511	4	2	616	1	
411		8	516	6	0	621	0	
416		4	521	5	0	626	0	
421		3	526	4	0	631	0	
426		5	531	2	0	636	1	
431		6	536	6	1	641	0	
436	1	5	541	8	0	646	1	
441	0	6	546	5	0			
446	1	7	551	5	0			
451	0	3	556	6	0			
456	0	5	561	1	1		100	96

	Female	Male	Difference	X
Mean and P E (mms)	528 7 ± 2 70	447 4 ± 2 50	+ 81 3 ± 3 68	
S D and P E (mms)	39 9 ± 1 91	36 4 ± 1 77	+ 3 6 ± 2 60	118 1
C V and P E (per cent)	7 6 ± 0 36	8 1 ± 0 40	— 0 6 ± 0 54	

Table CXXVIII

Girth of thigh, least

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
271—275 9		1	351	9	6	431	1	
276	1	0	356	7	5	436	0	
281	0	0	361	1	2	441	0	
286	2	2	366	0	2	446	0	
291	3	1	371	1	1	451	0	
296	2	4	376	0	1	456	0	
301	6	2	381	0		461	0	
306	4	7	386	1		466	0	
311	7	4	391	0		471	0	
316	8	8	396	2		476	0	
321	7	10	401	0		481	0	
326	5	9	406	0		486	0	
331	7	10	411	1		491	0	
336	10	8	416	2		496	1	
341	3	7	421	0				
346	6	6	426	0			100	96

	Female	Male	Difference	X
Mean and P E (mms)	337 ± 2 26	329 8 ± 1 50	+ 7 2 ± 2 71	102 2
S D and P E (mms)	33 5 ± 1 60	21 8 ± 1 06	+ 11 3 ± 1 92	
C V and P E (per cent)	9 9 ± 0 47	6 6 ± 0 33	+ 3 3 ± 0 58	

Table CXXIX

Height, Sphenion

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
36—40 9	2		61	17	22	86		1
41	2		66	12	29	91		1
46	10	1	71	12	22			
51	15	1	76	2	10			
56	27	8	81	1	5		100	100

	Female	Male	Difference	X
Mean and P E (mms)	59 6 ± 0 59	69 6 ± 0 49	— 10 ± 0 77	85 5
S D and P E (mms)	8 8 ± 0 42	7 3 ± 0 35	+ 1 5 ± 0 55	
C V and P E (per cent)	14 8 ± 0 72	10 5 ± 0 50	+ 4 3 ± 0 88	

Table CXXX

$$\frac{\text{Lower leg} \times 100}{\text{Thigh}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
62—63 9		1	78	12	15	94	3	0
64		0	80	12	14	96	0	1
66		0	82	17	16	98	0	0
68		0	84	13	21	100	1	0
70		0	86	6	5	102	0	0
72	6	4	88	9	4	104	1	1
74	5	7	90	4	3			
76	8	7	92	3	0			
							100	99

	Female	Male	Difference	X
Mean and P E	83 2 ± 0 41	82 ± 0 36	+ 1 2 ± 0 55	101 5
S D and P E	6 1 ± 0 29	5 4 ± 0 26	+ 0 7 ± 0 39	
C V and P E	7 3 ± 0 35	6 5 ± 0 31	+ 0 8 ± 0 47	

Table CXXXI

$$\frac{\text{Upper arm} + \text{Forearm} \times 100}{\text{Length of thigh} + \text{Length of lower leg}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
56—56 9	1		65	13	6	74		0
57	0		66	14	17	75		0
58	1		67	13	17	76		0
59	1		68	16	17	77		1
60	1	1	69	12	17	78		1
61	1	0	70	3	7	79		1
62	1	3	71	2	2	80		0
63	7	1	72	1	2	81		1
64	13	4	73		1			
							100	99

	Female	Male	Difference	X
Mean and P F	66 6 ± 0 18	68 3 ± 0 21	− 1 7 ± 0 28	97 5
S D and P E	4 4 ± 0 13	3 1 ± 0 15	+ 1 3 ± 0 22	
C V and P E	4 0 ± 0 19	4 6 ± 0 22	− 0 6 ± 0 29	

Table CXXXII

Forearm × 100 / Length of lower leg

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
48—49 9	1		64	7	17	80		0
50	0		66	17	28	82		0
52	0	1	68	10	16	84		0
54	3	0	70	11	9	86		0
56	4	1	72		3	88		0
58	6	3	74		1	90		1
60	20	6	76		1			
62	21	12	78		1		100	100

	Female	Male	Difference	X
Mean and P E	64 0 ± 0 29	66 7 ± 0 31	− 2 7 ± 0 43	96
S D and P E	4 3 ± 0 21	4 6 ± 0 22	− 0 3 ± 0 30	
C V and P E	6 7 ± 0 32	6 9 ± 0 33	− 0 2 ± 0 46	

Table CXXXIII

Girth of calf

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
256—260 9	1		306	8	9	356	2	1
261	1		311	6	14	361	1	
266	1	1	316	9	14	366	0	
271	3	2	321	4	7	371	1	
276	3	1	326	6	8	376	0	
281	9	0	331	5	2	381	0	
286	6	4	336	1	7	386	0	
291	11	5	341	6	8	391	1	
296	4	6	346	1	3			
301	7	4	351	3	1		100	97

	Female	Male	Difference	X
Mean and P E (mms)	309 8 ± 1 73	316 3 ± 0 69	− 6 5 ± 1 86	97 8
S D and P E (mms)	25 6 ± 1 22	10 1 ± 0 49	+ 15 6 ± 1 32	
C V and P E (per cent)	8 1 ± 0 39	3 2 ± 0 15	+ 4 9 ± 0 42	

Table CXXXIV

$$\frac{\text{Girth of calf} \times 100}{\text{Length of lower leg}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
70—71 9	2	3	86	10	11	102	3	2
72	0	1	88	8	9	104	3	
74	0	10	90	11	5	106	3	
76	1	3	92	12	3	108	0	
78	5	8	94	10	6	110	0	
80	7	9	96	2	2	112	1	
82	9	10	98	2	1	114	1	
84	6	13	100	4	1			
							100	97

	Female	Male	Difference	X
Mean and P E	90 6 ± 0 56	84 6 ± 0 48	+ 6 ± 0 74	
S D and P E	8 4 ± 0 40	7 1 ± 0 34	+ 1 3 ± 0 53	107
C V and P E	9 2 ± 0 44	8 4 ± 0 41	+ 0 9 ± 0 60	

Table CXXXV

$$\frac{\text{Girth of calf} \times 100}{\text{Maximum girth of thigh}} \text{ (males only)}$$

Class per cent	Z Male	Class per cent	Z Male	Class per cent	Z Male	Class per cent	Z Male
56—57 9	1	62	10	68	22	74	4
58	1	64	17	70	9	76	2
60	4	66	18	72	6	78	1
							95

Mean and P E	67 7 ± 0 28
S D and P E	4 ± 0 20
C V and P E	5 9 ± 0 29

TABLE CXXXVI

Girth of ankle, least

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
161—165 9		1	201	11	16	241		0
166	2	1	206	3	14	246		0
171	9	1	211	5	4	251		0
176	7	5	216	1	2	256		0
181	18	7	221	0	2	261		1
186	11	11	226	1	0			
191	13	12	231	1	1			
196	17	18	236	1	2		100	98

	Female	Male	Difference	X
Mean and P E (mms)	191 7 ± 0 91	198 4 ± 1	− 6 7 ± 1 35	96 5
S D and P E (mms)	13 5 ± 0 64	14 7 ± 0 70	− 1 2 ± 0 95	
C V and P E (per cent)	7 0 ± 0 34	7 4 ± 0 36	− 0 4 ± 0 49	

TABLE CXXXVII

$$\frac{\textit{Girth of ankle} \times 100}{\textit{Girth of calf}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
53—54 9	1		65	3	13	77		0
55	4	1	67	8	2	79		0
57	8	5	69	2	1	81		0
59	23	23	71		0	83		1
61	31	22	73		1			
63	20	27	75		1		100	97

	Female	Male	Difference	X
Mean and P E	62 1 ± 0 21	63 0 ± 0 26	− 1 ± 0 33	98 5
S D and P E	3 1 ± 0 15	3 8 ± 0 18	− 0 7 ± 0 25	
C V and P E	4 9 ± 0 24	6 ± 0 29	+ 1 1 ± 0 37	

Table CXXXVIII

Length of entire arm with hand × 100 / Entire leg

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
71—71 9	1		78	2	5	85	7	14
72	0		79	6	2	86	6	4
73	0		80	9	7	87	5	5
74	1		81	12	8	88	4	1
75	0	1	82	12	14	89	2	1
76	0	1	83	12	19			
77	4	1	84	17	16		100	99

	Female	Male	Difference	X
Mean and P E	83 1 ± 0 21	83 3 ± 0 17	− 0 23 ± 0 27	99 7
S D and P E	3 1 ± 0 15	2 6 ± 0 12	+ 0 6 ± 0 19	
C V and P E	3 8 ± 0 18	3 1 ± 0 15	+ 0 7 ± 0 23	

Table CXXIX

Length of foot

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
201—205 9	1		231	20	7	261		11
206	3		236	8	11	266		7
211	8		241	6	19	271		1
216	17		246	2	11	276		3
221	16		251		13			
226	19	2	256		14		100	99

	Female	Male	Difference	X
Mean and P E (mms)	226 1 ± 0 63	250 3 ± 0 82	− 24 3 ± 1 09	90 1
S D and P E (mms)	9 4 ± 0 45	12 2 ± 0 58	− 2 8 ± 0 73	
C V and P E (per cent)	4 1 ± 0 20	4 8 ± 0 23	− 0 7 ± 0 36	

Table CXL

Foot length × 100 / Lower leg

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
56—57 9	1		64	25	19	72	5	7
58	3	3	66	20	23	74	4	0
60	8	7	68	13	13	76		1
62	12	9	70	9	16			
							100	98

	Female	Male	Difference	X
Mean and P E	66 4 ± 0 26	67 0 ± 0 25	− 0 6 ± 0 36	99
S D and P E	3 8 ± 0 18	3 6 ± 0 17	+ 0 2 ± 0 25	
C V and P E	5 7 ± 0 27	5 4 ± 0 26	+ 0 3 ± 0 38	

Table CXLI

Breadth of foot

Class mms.	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
71—75 9	1		86	27	18	101		6
76	21		91	5	46	106		1
81	46	8	96		20		100	99

	Female	Male	Difference	X
Mean and P E (mms)	83 2 ± 0 27	92 2 ± 0 37	− 9 ± 0 45	90 2
S D and P E (mms)	3 9 ± 0 19	5 4 ± 0 26	− 1 5 ± 0 32	
C V and P E (per cent)	4 7 ± 0 24	5 9 ± 0 28	− 1 1 ± 0 37	

Table CXLII

$$\frac{\text{Foot breadth} \times 100}{\text{Foot length}}$$

Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male	Class per cent	Z Female	Z Male
33—33 9	2	1	39	8	8	45		0
34	9	8	40	5	4	46		0
35	22	18	41	1	2	47		0
36	20	23	42		1	48		1
37	20	14	43		0			
38	13	19	44		0		100	99

	Female	Male	Difference	X
Mean and P E	37 0 ± 0 12	37 3 ± 0 14	− 0 3 ± 0 18	
S D and P E	1 7 ± 0 08	2 1 ± 0 10	− 0 4 ± 0 13	99 1
C V and P E	4 6 ± 0 22	5 7 ± 0 27	− 1 0 + 0 11	

Table CXLIII

Bicondylar diameter at elbow

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
46—50 9	8		56	40	14	66		26
51	51	1	61	1	56	71		2
							100	99

	Female	Male	Difference	X
Mean and P E (mms)	54 2 ± 0 19	63 2 ± 0 20	− 9 ± 0 28	
S D and P E (mms)	2 8 ± 0 15	2 9 ± 0 14	− 0 1 ± 0 23	85 6
C V and P E (per cent)	5 2 ± 0 25	4 6 ± 0 22	+ 0 6 ± 0 34	

TABLE CXLIV

Bistyloid diameter at wrist

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
31—35 9	1		41	71	10	51	1	25
36	13	1	46	11	60	56		3
							100	99

	Female	Male	Difference	X
Mean and P E (mms)	42 4 ± 0 17	48 5 ± 0 22	− 6 1 ± 0 27	
S D and P E (mms)	2 5 ± 0 12	3 2 ± 0 15	− 0 7 ± 0 19	87 5
C V and P E (per cent)	5 8 ± 0 28	6 6 ± 0 32	− 0 8 ± 0 42	

TABLE CXLV

Bicondylar diameter at knee

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
66—70 9	1		81	36	25	96	1	1
71	8		86	14	47			
76	39	6	91	1	20		100	99

	Female	Male	Difference	X
Mean and P E (mms)	80 6 ± 0 31	86 8 ± 0 27	− 6 2 ± 0 40	
S D and P E (mms)	4 6 ± 0 22	4 ± 0 19	+ 0 6 ± 0 29	92 8
C V and P E (per cent)	5 7 ± 0 27	4 6 ± 0 22	+ 1 1 ± 0 35	

TABLE CXLVI

Bimalleolar diameter, ankle

Class mms	Z Female	Z Male	Class mms	Z Female	Z Male	Class mms	Z Female	Z Male
56—60 9	32	2	66	6	54	76		2
61	61	13	71	1	28		100	99

	Female	Male	Difference	X
Mean and P E (mms)	61 3 ± 0 18	68 3 ± 0 24	− 7 ± 0 30	
S D and P E (mms)	2 7 ± 0 13	3 5 ± 0 17	− 0 7 ± 0 21	89 6
C V and P E (per cent)	4 5 ± 0 21	5 1 ± 0 24	− 0 6 ± 0 32	

(*b*) SUMMARY AND DISCUSSIONS OF THE APPENDICULAR MEASUREMENTS

Measurements of the male group

Length of arm stretch is found to be 1734 3 mm (Table C) Arm stretch to total height relation is 104 4 per cent (Table CI) Height radial is 1035 mm (Table CII) Girth of upper arm is 270 9 mm (Table CIII) and its relation to the length of the upper arm is 85 4 per cent (Table CIV) Girth across contracted biceps is 268 3 mm (Table CV), lesser than the greatest girth of the arm Height Stylion is 783 5 mm (Table CVI), and the relation of lower arm to the upper arm is 76 9 per cent (Table CVII) Greatest girth of the forearm is 239 6 mm (Table CVIII), while the relation of the girth to the length of forearm is 96 5 per cent (Table CIX), and that of the girth of forearm to the girth of the upper arm is 88 9 per cent (Table CX) Height Dactylion is 597 6 mm (Table CXI) Length of total arm minus hand, to total height is 34 2 per cent (Table CXII) and upper arm length to total length of the arm is 42 2 per cent (Table CXIII), and lower arm length to the total arm is 33 3 per cent (Table CXIV) Girth of the wrist is 155 8 mm

(Table CXV) and it is 57 9 per cent of the girth of the upper arm (Table CXVI) and 65 2 per cent of the girth of the forearm (Table CXVII) Length of the hand is 180 9 mm (Table CXVIII), and is 72 6 per cent of the forearm length (Table CXIX) Hand breadth 78 1 mm (Table CXX) is 43 6 per cent of the hand length (Table CXXI)

Height Trochanterian is 880 mm (Table CXXII) Height Tibiale is 445 1 mm (Table CXXIII) Upper arm is 69 4 per cent of the length of the thigh (Table CXXIV) Maximum girth of thigh is 469 5 mm (Table CXXV) and is 102 7 per cent of the length of the thigh (Table CXXVI) Girth in the middle of thigh is 447 4 mm (Table CXXVII) Least girth of the thigh is 329 8 mm (Table CXXVIII) Spherion height is 69 6 mm (Table CXXIX) Lower leg is 82 per cent of the thigh (Table CXXX) Total arm without hand is 68 3 per cent of the total leg without foot (Table CXXXI) Forearm is 66 7 per cent of the lower leg (Table CXXXII) Girth of calf is 316 3 mm (Table CXXXIII), and is 84 6 per cent of the length of the lower leg (Table CXXXIV) and 67 7 per cent of the maximum girth of the thigh (Table CXXXV) Girth at ankle is 198 4 mm (Table CXXXVI) which is 63 per cent of the girth of calf (Table CXXXVII) Entire arm is 83 3 per cent of the entire leg (Table CXXXVIII) Length of the foot is 250 3 mm (Table CXXXIX), which is 67 per cent of the lower leg (Table CXL) Foot breadth 92 2 mm (Table CXLI) is 37 3 per cent of the foot length (Table CXLII) Bicondylar diameter at elbow is 63 2 mm (Table CXLIII) Bistyloid at wrist is 48 5 mm (Table CXLIV), bicondylar at knee is 86 8 mm (Table CXLV), and bimalleolar at ankle is 68 3 mm (Table CXLVI)

The least coefficient of variation 3 17 per cent is about the girth of calf (Table CXXXIII), the greatest variation 10 5 per cent being about the height Spherion (Table CXXIX) in case of averages of measurements

In indices, the least variation 2 31 per cent is about arm stretch total stature index (Table CI) and the greatest 10 14 per cent about the girth length index of thigh (Table CXXVII)

In the absence of any suitable material no comparisons could be made as regards the above results

Measurements of the female group

The arm stretch is 1553 4 mm (Table C) The American women have 1621–1647 mm Arm stretch is 102 2 per cent of the total height (Table CI) and the Americans' is 99 4 per cent Height radial is 944 2 mm (Table CII), while Americans' is 1024 and 1035 mm , according to two different studies Greatest girth of the upper arm is 245 2 mm (Table CIII), while Americans have 240 4 mm Girth of the upper arm is 87 2 per cent of its length (Table CIV) Girth across contracted biceps is 246 9 mm (Table CV) which is greater by a millimetre than the greatest girth

Height Stylion is 725 5 mm (Table CVI), while Americans' is 800 65 mm Lower arm is 77 6 per cent of the upper arm (Table CVII), while Americans' index is 76 3 and 75 7 per cent in two studies Greatest girth of the forearm is 215 mm (Table CVIII), while Americans' is 229 35 mm Girth of the forearm is 98 8 per cent of its length (Table CIX) Girth of the forearm is 87 8 per cent of that of the upper arm (Table CX) Height Dactylion is 550 7 mm (Table CXI), while the Americans have 615 7 and 613 8 mm Total arm minus hand is 33 3 per cent of the total stature (Table CXII) while Americans' is 31 35 and 32 78 per cent Upper arm is 41 9 per cent of the total arm with hand (Table CXIII) American figure is 56 74 per cent in one group (?) and 41 6 per cent in another group Lower arm is 32 47 per cent of the total arm with hand (Table CXIV), and Americans have 43 13 (?) and 31 13 per cent in two groups Our wrist girth is 139 9 mm (Table CXV), and is 57 6 per cent of the girth of the upper arm (Table CXVI), and 65 4 per cent of the girth of the forearm (Table CX) Hand length is 168 2 mm (Table CXVIII), while the studies in America give 187 4, 177 6 and 186 4 mm as their lengths Hand length is 76 9 per cent of the forearm (Table CXIX) Hand breadth is 70 4 mm (Table CXX), and is 42 1 per cent of its length (Table CXXI) American hand breadth is 79 83 and 79 4 mm in two studies, and hand index is 42 47 and 42 89 per cent Height Trochanterian is 798 2 mm (Table CXXII) Height Tibiale is 402 4 mm (Table CXXIII) Girth in the middle of the thigh is 528 7 mm (Table CXXVII) Least girth of thigh is 336 9 mm (Table CXXVIII) Spherion height 59 55 (Table CXXIX) American internal malleolar height is 77 56 and 78 69 mm in two groups Lower leg is 83 2 per cent of the thigh (Table CXXX) Total arm minus hand is 66 6 per cent of the total leg minus height Spherion (Table CXXXI) Length of the forearm is 64 per cent of the length of the lower leg (Table CXXXII) Calf girth is 309 8 mm (Table CXXX) and is 90 6 per cent of the leg length (Table CXXXIV) American calf girth is 336 4 and 322 8 mm in two studies Girth of ankle is 191 6 mm (Table CXXXVI) which is 62 per cent of the girth of the calf (Table CXXXVII) American ankle girth is 208 5 and 197 8 mm in two studies Length of the total arm is 83 per cent of the total leg (Table CXXXVIII) Length of the foot is 226 mm (Table CXXXIX) and is 66 4 per cent of the length of the lower leg (Table CXL) American foot length is 236 8, 240 7 and 236 4 mm in three studies Breadth of foot is 83 2 mm (Table CXLI), and is 36 9 per cent of the length (Table CXLII) American foot breadth is 83 17 mm and is 34 81 and 36 52 per cent of the length in two different studies Bicondylar diameter at elbow is 54 2 mm (Table CXLIII) bistyloid at wrist 42 4 mm (Table CXLIV), bicondylar at knee 80 5 mm (Table CXLV), and bimalleolar at ankle 61 3 mm (Table CXLVI)

The least variation of subjects about the mean is 3 85 per cent and it is in case of arm stretch (Table C) The greatest variation 14 8 per cent (Table CXXIX) is about the height Spherion

In indices, the least variation, 2 36 per cent, is about the arm stretch total stature index (Table CI) The greatest, 12 1 per cent, is about the girth length index of the upper arm (Table CIV)

Comparison of male and female standards (Fig 3)

Height Spherion and Bicondylar diameter at elbow of the female group are 85 per cent of those of the male group (Tables CXXIX and CXLIII) Bistyloid diameter at knee of the female is 87 per cent of the male one (Table CXLIV) Standards of arm stretch, greatest girth of forearm, least girth at wrist, and bimalleolar diameter at ankle of the ladies are 89 per cent each of the same of the men (Tables C, CVIII, CXV and CXLVI) Greatest girth of the upper arm, hand breadth, trochanteric height, tibiale height, foot length, and foot breadth happen to be 90 per cent of the male standards (Tables CIII, CXX, CXXII, CXXIII, CXXXIX and CXLI) Radial height and girth across contracted biceps of the ladies are found to be 91 per cent of the male standards (Tables CII and CV) Height Stylion, height Dactylion, and Bicondylar diameter at knee are 92 per cent of the same of the men (Tables CVI, CXI and CXLV) Hand length is 93 per cent (Table CXVIII) Girth of ankle is 96 per cent (Table CXXXVI) girth of calf 97 per cent (Table CXXXIII), least girth of thigh 102 per cent (Table CXXVIII) and girth of the middle of the thigh 118 per cent of the male standards (Table CXXVII)

Hand and forearm length indices are 96 per cent of the male ones (Tables CXXI and CXXXII) Total arm minus hand to total height, lower arm to total hand, total arm minus hand to total leg minus Spherion indices are 97 per cent (Tables CXII, CXIV and CXXXI) Arm stretch to total height, forearm girth to upper arm girth, and girth at ankle to that of calf indices are 98 per cent (Tables CI, CX and CXXXVII) Upper arm to total arm, girth of wrist to girth of upper arm, upper arm length to thigh length, entire arm to entire leg, foot length to lower leg and foot breadth to length indices are 99 per cent of the same of the male group (Tables CXIII, CXVI, CXXIV, CXXXVIII, CXL and CXLII) Lower to upper arm and wrist girth to forearm girth indices are the same as men's (Tables CVII and CXVII) Lower leg to thigh index is 101 per cent (Table CI). Girth length index of upper arm is 102 per cent (Table CIV) Girth length index of forearm is 104 per cent (Table CIX) Hand length to forearm index is 106 per cent (Table CXIX), and girth length index of lower leg is 107 per cent of the male one (Table CXXXIV)

Aggregate average of the appendicular measurements of the female is 92 54 per cent of the male appendages

Aggregate average of appendicular indices is 100 per cent of the male indices

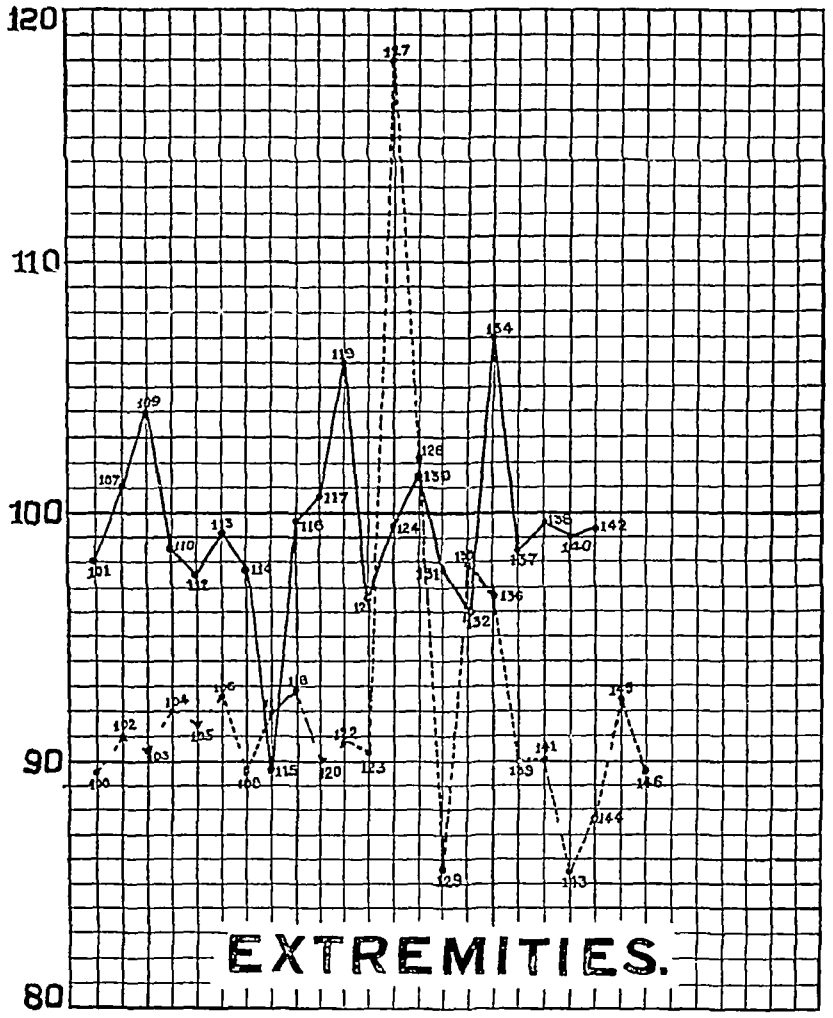

FIG 3

Comparison of appendicular study of males and females

A graph showing the comparison of the standards of appendicular measurements and indices The standards of the femal measurements and indices have been reduced to the percentage of the male standards Thus all male standards represent the line 100 The numbered dots along the broken line indicate the table numbers of measurements of female group, and those along the unbroke line indicate indices of the same The numbers refer to table numbers

Dot numbers along the broken line (measurements)

100 Arm stretch
102 Ht Radial
103 Max G up arm.
105 G contr biceps
106 Ht Stylion
108 Max G forearm
111 Ht Dactylion
115 Lst G wrist
118 L of hand
120 B of hand
122 Ht Trochant
123 Ht Tibiale
127 G mid thigh
128 G thigh lst
129 Ht Spherion
133 G of calf
136 Ankle G lst
139 Foot length
141 Foot breadth
143 Bicond D elbow
144 Bistyloid D wrist
145 Bicond D knee
146 Bi malleol D ankle

Dot numbers along the unbroken line (indices)

101 Arm stretch / Tot stature
104 Upper arm girth / L of up arm
107 Lower arm / Upper arm
109 Forearm G / Forearm L
110 Forearm G / Upper arm G
112 Acrom *minus* stylion / Tot. stature
113 Upper arm / Tot arm—hand
114 Lower arm / Tot arm—hand
116 Wrist girth / Upper arm G
117 Wrist girth / Forearm girth
119 Hand length / Forearm L.
121 Hand breadth / Hand length
124 Upper arm / L of thigh
130 Lower leg / L of thigh
131 Upper arm—fore arm / Thigh leg
132 Forearm / Lower leg
134 Calf girth / L of lr leg
135 Calf girth / Max. thigh G.
137 Ankle girth / Calf girth
138 Tot arm—hand / Tot leg
140 Length of foot / Lower leg
142 Foot breadth / Foot length

J, MR

Study of Skin, Eyes and Hair.

TABLE CXLVII

Skin

Colour	Female	Male	Colour	Female	Male
Brown	59	59	Fair brown	4	5
Medium brown	10	16	Brownish		2
Light brown	6		Blackish brown		1
Dark brown	2	6	White	9	
Black brown	2		Unrecorded		1
Pale brown	6	9			
Whitish brown	2	1		100	100

TABLE CXLVIII

Eyes

Colour	Female	Male	Colour	Female	Male
Brown	31	47	Brownish black		12
Dark brown	42	24	Green	1	
Pale brown	2		Gray	2	
Medium brown	3	2	Light yellow		1
Light brown	3		Greenish brown		1
Blackish brown	9	3	Yellowish green		1
Yellowish brown	2	4	Unrecorded	4	2
Brownish	1				
Black		3		100	100

TABLE CXLIX

Hair

Colour	Female	Male	Colour	Female	Male
Black	84	94	Light brown	1	
Brownish black	4	3	Brownish	1	1
Brown	5		Unrecorded		1
Blackish brown	1				
Dark brown	4	1		100	100

We are now engaged in studying the community of fishermen and fisherwomen along the coast of Bombay

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A table of summary of anthropometric data from the Bombay Presidency

Serial number	Names of tribes or castes	Numbers of subjects	Cephalic length	Cephalic breadth	Cephalic index	Nasal height	Nasal breadth	Nasal index	Height from vertex to root of nose	Height from vertex to tragus of ear	Height from vertex to chin	Cephalic volume
1	Khandesh Bhil	100	182 9	132 6	72 4	41 1	39 0	94 8	103 8	117 5	208 5	142 3
2	Kathari	100	178 8	133 0	74 3	41 0	38 7	87 9	94 1	125 3	200 1	145 7
3	High Caste Maratha	100	181 6	140 9	76 3	52 0	37 0	71 1	105 6	131 4	216 7	152 3
4	Maratha Lohar	15	180 2	137 8	76 4	47 6	35 8	75 2	105 6	132 4	214 3	150 1
5	Bene Israil	60	184 1	141 1	76 6	50 2	36 5	72 7	104 5	138 9	220 1	154 8
6	Southern Jain Pancham	30	183 2	140 9	76 9	51 4	39 6	77 0	107 4	128 4	217 7	150 7
7	Deshastha Brahmin	100	185 4	142 9	77 0	48 9	38 8	79 3	109 4	136 3	219 5	154 8
8	Mahar	100	181 6	140 0	77 0	47 2	38 7	81 9	104 7	130 1	212 5	150 6
9	Kokanastha Brahmin	100	186 4	144 2	77 3	49 3	37 8	76 6	109 4	134 3	218 2	155 0
10	Kunbi	100	180 1	139 4	77 4	47 9	37 9	79 1	100 9	128 9	209 0	149 5
11	Tambat Kasar	59	181 8	140 8	77 4	45 7	36 8	80 5	102 3	134 7	211 3	152 4
12	Koli (Son)	100	185 0	143 4	77 5	49 6	37 9	76 4	102 0	129 2	213 4	152 5
13	Namdev Shimpi	100	178 5	139 5	78 1	45 1	36 1	80 0	102 5	135 8	213 8	151 3
14	Thakur	90	174 0	136 2	78 2	41 3	36 5	88 3	105 3	132 1	210 0	147 4
15	Maratha Ghati	100	181 3	142 1	78 3	47 8	38 2	79 9	103 9	132 2	211 5	151 9
16	Dakshni Vanjari	20	178 6	140 6	78 7	47 3	37 6	70 4	107 5	121 4	213 1	146 9
17	Vaidu	12	179 9	142 0	78 9	46 5	36 4	78 2	108 2	130 5	216 3	150 8
18	Vani or Bania	127	183 8	145 2	78 9	49 9	37 8	75 7	103 8	130 5	213 6	153 2
19	Shenvi or Saraswat Brahmin	100	186 2	147 1	79 0	50 3	37 6	74 7	103 1	134 1	215 0	155 8
20	Nagar Brahmin	100	184 4	147 1	79 7	50 7	37 1	73 1	107 0	133 6	216 6	155 0
21	Prabhu	100	184 2	147 2	79 9	50 1	38 0	75 8	100 8	133 2	212 8	154 9

collected under the auspices of the Ethnological Society of India in 1906

Maximum bizygomatic breadth	Nasio mental length	Facial index	Facial angle (Cuvier)	External orbital breadth	Bi orbito nasal arc	Orbito nasal index	Bigonial breadth	Height, Standing	Height, Sitting	Height, Kneeling	Height to chin	Height to external	Bi iliac crest breadth
128 6	104 7	122 8	69 0	102 9	125 4	121 8	100 9	164 9	83 2	121 3	144 6	137 2	251 6
126 0	106 2	118 6	63 6	107 8	121 2	112 4	99 3	158 4	78 4	116 8	138 4	129 5	239 2
133 9	111 0	120 6	71 1	106 8	133 1	124 6	104 7	168 1	86 1	124 3	146 5	139 0	269 0
130 9	108 6	120 5	71 0	103 3	123 8	122 2	100 6	159 9	82 9	118 6	139 8	131 7	250 4
130 7	115 6	113 0	70 8	106 9	135 1	126 3	101 1	166 3	86 0	123 0	145 4	137 4	267 8
130 6	110 3	118 4	69 3	104 5	131 7	126 0	101 5	162 8	83 1	120 2	141 8	134 9	257 1
132 5	110 3	120 1	68 3	116 6	135 6	116 2	101 4	164 2	84 9	121 8	141 9	134 1	256 9
131 7	107 7	122 2	66 1	113 9	130 6	114 6	104 9	163 4	81 1	119 8	143 2	134 0	251 4
132 4	108 8	121 6	68 6	116 0	134 2	115 6	99 0	165 5	85 1	122 5	144 0	135 5	256 2
130 0	108 0	120 3	66 4	113 2	129 5	114 3	102 3	160 0	80 3	117 4	138 8	131 0	246 0
130 7	108 9	120 0	74 0	100 4	123 1	122 6	99 2	161 3	83 1	120 5	140 5	133 5	253 7
133 6	111 3	120 0	66 3	114 5	129 5	113 1	104 7	160 1	80 6	118 1	138 6	131 0	251 7
130 6	111 2	117 4	73 3	100 3	119 1	118 7	102 3	162 8	83 7	120 9	142 5	136 2	251 7
128 5	104 7	122 7	72 8	96 9	120 1	123 9	97 3	160 6	80 4	117 9	139 8	133 0	237 8
132 5	107 5	123 2	67 7	114 9	133 1	115 8	103 6	163 2	82 0	119 9	142 2	133 6	258 7
131 3	105 6	124 3	69 2	101 7	129 0	126 8	101 4	166 8	84 4	122 6	145 9	138 7	255 1
133 1	108 0	123 2		96 4	119 0	123 4	103 1	160 2	81 7	118 9	140 0	133 5	260 0
132 3	109 7	120 6	68 0	113 1	131 5	116 2	102 9	161 2	83 6	120 0	139 8	132 6	253 9
133 2	112 0	118 9	68 3	112 9	129 5	114 7	102 6	163 4	84 6	121 1	142 2	133 7	257 1
132 7	109 5	121 1	69 8	114 1	133 2	116 7	101 7	164 3	86 2	122 5	142 6	134 8	254 4
132 1	111 9	118 0	66 7	113 0	128 2	113 4	101 6	162 7	85 0	121 0	141 3	133 3	252 7

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OBSERVATIONS ON ŒDEMATOUS NEPHRITIS

BY

LIEUT.-COLONEL T A HUGHES, M A, M D, M R C P, D P H, I M S,

AND

KHAZAN SINGH MALIK, M Sc

(*From the Department of Clinical Medicine, King Edward Medical College, Lahore*)

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IN 1927 an account was published (Hughes and Shrivastava, 1927) of certain observations carried out on 16 cases of œdematous nephritis admitted to the Mayo Hospital, Lahore, and it was concluded, *inter alia*, that in all except one, or possibly two, inflammatory changes in the kidneys were, to a greater or less extent, responsible for the conditions observed During the last few years much work has been done by Addis (1925, 1928) and by Van Slyke and his associates (1930) on the nature of Bright's disease and on the natural evolution of the disease processes, and the introduction by these workers of the urea clearance test has provided a very reliable indicator of renal efficiency In the light of this work a further study has been made of the œdematous nephritis seen in the Punjab Twenty-five cases were investigated

The conditions usually denoted by the term Bright's disease are classified by Addis and by Van Slyke and his colleagues on the lines laid down by Volhard and Fahr (1914) and by Volhard (1918) These authors recognized three main types of nephropathy each with distinct clinical features and distinct primary histological changes In one type there is 'hæmaturia acute, intermittent or chronic, usually with hypertension and with nitrogen retention, frequent in the acute stage, regular in the advanced chronic stages' (Van Slyke *et al*, *loc cit*), in another there is a marked rise in blood-pressure which precedes any serious renal changes, and in the third there is pronounced œdema and proteinuria without hæmaturia or increased arterial pressure The corresponding characteristic histological changes are, respectively, (1) inflammatory destruction of the glomeruli, (2) thickening and obliteration of the small renal arteries, and (3) degeneration of the renal tubules,

especially the convoluted tubules In any type secondary changes may be found in the terminal stages at autopsy and two varieties of the primary change may co-exist in the same subject We reproduce from Van Slyke's paper a table giving in a condensed form the chief characteristic features of the three types The synonyms in parenthesis are those of Volhard and Fahr (*loc cit*)

Volhard and Fahr and Addis believed that it was possible from certain observations during the course of Bright's disease to deduce the general nature of the lesions present in the kidneys and this belief has been supported by the work of Van Slyke and others (*loc cit*) In the majority of our cases only those examinations were done which were necessary for this purpose All observations were made in the morning while the patients were fasting The only functional test used was the urea clearance test which was carried out according to the method of Moller, McIntosh and Van Slyke (1928) In this method the following data are determined, quantities of urine excreted during two successive hours, concentration of urea in each sample of urine and concentration of urea in the blood at about the middle of each hourly period The degree of hæmaturia and cylindruria was roughly determined by microscopic examination of the centrifuged urine The quantitative sediment count of Addis (1925) could not be done owing to the difficulty of getting the patients to take a dry diet for 24 hours The percentage of urinary protein was estimated by Esbach's or Aufrecht's method in the samples of urine passed during the test Total daily estimations of urinary protein were not carried out Blood-pressure measurements were made with the mercury manometer

The plasma proteins were estimated in two cases only, the method used being that of Hawk and Bergeim (1926) The work of Epstein (1917, 1922) and others has now so definitely established plasma protein deficit as the main, if not the only, cause of true renal œdema that routine estimation of these proteins was not considered necessary Starling (1895-96) showed that the plasma proteins exerted an osmotic pressure of about 30 mm Hg and pointed out the importance of this pressure in balancing the tendency of the hydraulic pressure in the capillaries to force fluid into the tissue spaces The effect of a reduction in plasma proteins in causing œdema is therefore obvious Subsequent work has confirmed Starling's observations The salts of the plasma can have little effect in controlling diffusion through the capillary walls as these salts themselves diffuse freely into the tissue spaces The approximate threshold values for the occurrence of œdema have been shown to be a plasma albumin content of 2 5 per cent, a total plasma protein content of 5 5 per cent and a plasma specific gravity of 1023 Plasma globulin has little influence in the tendency to œdema formations since it exerts only about $\frac{1}{4}$ the osmotic pressure of plasma albumin (Govaerts, 1925 and 1926) The effect of protein deficit may of course be modified by other factors Thus when the protein content is near the critical level the intake of salt may determine the presence or absence of œdema, although Moore and Van Slyke (1930) have shown

TABLE

Course of Bright's disease (Van Slyke et al)

Type of disease		Clinical course	Urine sediment (Addis)	Anatomical changes of kidneys
Hæmorrhagic (glomerulonephritis)		Acute onset Either heals, improves to a latent condition, or progresses through an intermediate chronic state, usually œdematous, with diminishing renal function, to terminate in uræmia	Red cells in varying numbers Blood, epithelial, and granular casts, in all stages except terminal Hyaline casts in all stages Broad 'renal failure' casts in terminal stage	Glomerular inflammation leading in terminal stage to nearly complete destruction Intracapillary and extracapillary forms of Fahr Also varying tubular degeneration and arterial changes
Non hæmorrhagic	Arteriosclerotic (nephrosclerosis)	Insidious onset Marked hypertension No œdema unless cardiac Death by cardiac failure, apoplexy or uræmia	Chiefly hyaline casts	Arterioles are diseased, with contracted lumina endarteritis, intimal hyperplasia, fatty degeneration, necrosis, in varying degree Varying proportions of glomeruli destroyed No marked tubular degeneration Necrosis of tubules may occur
	Degenerative (nephrosis, lipoid or amyloid)	Insidious onset Œdema, and proteinuria No hypertension May end in cure, death by intercurrent infection, or less frequently uræmia	Chiefly hyaline casts, few epithelial, fatty, granular, and waxy No blood casts Failure casts in cases with terminal uræmia Doubly refracting globules	Degenerated tubular epithelium Varying proportions of glomeruli may be destroyed, hyaline or amyloid In amyloid type arteriolar walls are more or less infiltrated by amyloid material

that entire disappearance of œdema is unusual when the proteins are below the threshold Other conditions that influence the production of œdema are infections and fevers, which probably act by increasing the permeability of the capillaries, and vomiting which, by causing desiccation, may exert an inhibitory effect Van Slyke *et al* (*loc cit*) found that in the early weeks of acute nephritis œdema may occur when the plasma proteins are normal in amount and they consider this œdema to be due to increased capillary permeability brought about by the toxins of the disease The œdema in the terminal stages of hæmorrhagic or arteriosclerotic nephritis may also be associated with a normal level of plasma proteins Such œdema is cardiac in origin

Cholesterol estimations were done by Mayer and Wardell's method (Mayer and Wardell, 1918) in four cases The values in all were above or at the upper limit of normal Hypercholestrolæmia is a recognized feature of the 'nephrotic syndrome' and is therefore found in the chronic active stage of hæmorrhagic nephritis and in degenerative nephritis Its significance is not understood but it seems to be due to a tendency to mobilize lipoids into the blood stream and may be related to malnutrition For the purpose of diagnosing the type of renal disease routine cholesterol estimations are not essential The degree of anæmia also was not determined as a routine procedure Although reduction of hæmoglobin is of frequent occurrence, especially in the hæmorrhagic and degenerative types, as a result of damage to the bone-marrow, it is not of diagnostic importance It does, however, influence the prognosis

The principles underlying the urea clearance test and the other observations on which diagnoses were based may be briefly set forth The urea clearance indicates the hypothetical quantity of blood which would have to be completely cleared of its urea in one minute to supply the amount of urea excreted in that time by the kidneys Austin, Stillman and Van Slyke (1921) showed that when in normal subjects the urinary volume is above 2 c c per minute (called the 'augmentation limit'), the clearance is independent of the volume and is calculated as $\frac{U\,V}{B}$ where U is the concentration of urea in the urine, B is the concentration of urea in the blood and V is the volume of the urine excreted in one minute This is called the maximal clearance and averages 75 c c per minute for normal adults When, however, the volume of urine is less than 2 c c per minute the rate of urea excretion falls with diminishing volume on an average in proportion to the square root of the volume The clearance under these circumstances is estimated for a standard urine output of 1 c c per minute, the formula used being $\frac{U}{B}\sqrt{V}$* This is known as the standard clearance and has an average value of 54 c c for normal

* For the development of this formula *see* Peters and Van Slyke (1931), page 346

adults Moller, McIntosh and Van Slyke (*loc cit*) found that the same percentages of average normal function are given by both clearances so that either formula can be used, depending on the minute output of urine From a comparison of post-mortem findings with terminal clearance values Van Slyke *et al* (*loc cit*) formed the opinion that 'in hæmorrhagic and degenerative nephritis one may interpret the blood urea clearance as a measure of the proportion of glomerular tissue functioning' and 'that it appears probable that in arteriosclerotic nephritis the fall in blood urea clearance is proportional to the decrease in renal blood flow rather than to the glomerular destruction' The urea clearance test was found by Van Slyke, McIntosh, Moller, Hannon and Johnston (1930) to be much a more sensitive indicator of renal efficiency than the blood urea (considered apart from urea excretion), the blood creatinine or the excretion of phenolsulphonephthalein In a large proportion of cases showing normal values for all these the urea clearance indicated only 40 per cent or less of normal renal function The clearance test is also much more sensitive than the urea concentration test which neglects the blood urea altogether In all the cases examined by us except one (No 13) the urine volumes were below 2 c c per minute

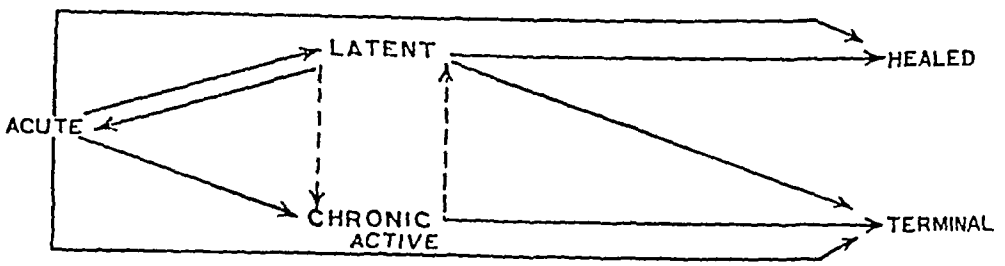
The importance of hæmaturia in differentiating the hæmorrhagic type of nephritis from the degenerative and arteriosclerotic types has been emphasized by Addis (*loc cit*) and by Van Slyke *et al* (*loc cit*) In acute hæmorrhagic nephritis blood is usually present in the urine in macroscopic amounts but in the chronic stages of the disease microscopic examination after centrifuging is essential Addis puts his patients on a dry diet for 24 hours, centrifuges the concentrated urine secreted during the last 12 hours of this period and counts the red cells and casts in the sediment Normal urine contains from 0 to 425,000 red cells in such a 12 hours output As already mentioned it was not feasible to carry out this procedure in the case of our patients but the results obtained in the ordinary way were found to be of value Van Slyke *et al* (*loc cit*) point out that during the latent and chronic active stages of hæmorrhagic nephritis the urine may be free from red blood cells

The degree of proteinuria is to some extent an indication of the plasma protein deficit although other factors, notably malnutrition, play a part in causing hypoproteinæmia A heavy proteinuria and marked reduction of the plasma proteins are almost constantly associated with degenerative disease of the kidneys whether hæmorrhagic or not A reduction in the urinary protein usually occurs as the disease passes into the terminal stage and this may be accompanied by a rise in blood-pressure A high blood-pressure with a trace of protein in the urine is the usual finding in arteriosclerotic cases

Arterial hypertension in renal disease is found in both the arteriosclerotic and hæmorrhagic forms In the former it precedes any serious renal lesions while in the latter it is the result of the renal disease In the hæmorrhagic disease it is

most usually found in the active stages (acute and chronic) and in the terminal stage although many cases never have a rise of blood-pressure at all A low pressure in the terminal stage does not improve the prognosis A fall in blood-pressure is sometimes seen at the very end in cases that have had hypertension and probably indicates failure of the heart muscle Six of our cases showed a blood-pressure level above normal Of these 4 were adjudged to be primarily arteriosclerotic in type and 2 primarily hæmorrhagic

Detailed accounts of the 3 varieties of renal disease are given by Van Slyke *et al* (*loc cit*) and by Peters and Van Slyke (1931) and the description which follows is based on these accounts The course of hæmorrhagic nephritis with acute onset is represented in the diagram adapted by the former authors from Addis (1925) —



The more usual changes are represented by the arrows with unbroken shafts As already mentioned, œdema may occur in the acute stage without any diminution in the plasma proteins and is then probably of toxic origin The diminution of renal efficiency during the first two months does not seem to affect the prognosis but if a low urea clearance does not show signs of returning steadily to normal within 4 months from the onset the outlook is unfavourable The degree of hæmaturia, of proteinuria and of hypertension attained during the acute stage are apparently not related to the prognosis but a low level of the plasma albumin is some indication of the ultimate outcome of the disease The prognosis is better when the plasma albumin is above 2 2 per cent and the total protein above 5 5 per cent In the latent stage the subjective symptoms and functional disturbances practically disappear There may be slight hæmaturia, a small degree of proteinuria or some reduction in the urea clearance Sometimes a small deficit in the plasma albumin persists or the blood-pressure may be somewhat above normal In certain cases the remission is so complete and occurs so quickly that cure may be said to have taken place, but others remain latent for years and eventually end in uræmia The chronic active stage resembles the degenerative type of the disease in presenting the ' nephrotic syndrome ' but there is a greater degree of hæmaturia, impaired renal function and often a definite history of an acute attack Some

cases progress so rapidly that they may be said to pass directly from the acute to the terminal stage but in general the chronic active stage lasts from 6 months to 2 years When red blood cells are present in only small numbers in the urine or are completely absent, as they may be, during this stage its differentiation from degenerative nephritis may be very difficult The urea clearance test is not always of value, as renal function may be considerably diminished in the latter stages of pure 'nephrosis' If hypertension is present it indicates the hæmorrhagic type of disease The terminal stage of hæmorrhagic nephritis is considered by Van Slyke *et al* to be reached when the urea clearance has fallen to 20 per cent of normal This stage usually lasts about one year The 'nephrotic syndrome' may persist unchanged to the end but it often gets less and may almost disappear Under the latter circumstances the patient may actually feel better for a time In many cases a marked rise in B P occurs and cardiac œdema may set in before the end The urine always contains red blood cells in considerable numbers

The characteristic pathological change in hæmorrhagic nephritis is diffuse disease of the glomeruli which may result in complete destruction of almost all the secreting units There are inflammatory changes in the tufts resulting in increase in endothelial cells, leucocytic infiltration, lobulation and eventually hyaline necrosis Bowman's capsule may be thickened and its lining endothelium proliferated or the tuft and capsule may become adherent The tubules, especially the convoluted tubules, show more or less degeneration of a lipoid nature The amount of anatomical shrinkage of the kidney depends on the duration of the disease rather than in the degree to which renal function is reduced In cases that pass directly from the acute to the terminal stage the kidneys are large, soft and pale The renal architecture is well preserved but the glomeruli present extensive changes When, on the other hand, the disease has run a more or less chronic course there is a greater or less degree of fibrous shrinkage resulting, in terminal cases, in the typical 'small white' kidney In subacute cases the kidneys are firmer than normal with little or no shrinkage and the distinction between cortex and medulla is still obvious Marked degeneration of the tubules is typical of the chronic active stage

Addis (1931) has recently pointed out that hæmorrhagic nephritis may start and progress so insidiously that the disease is not noticed by the patient until it is well established These cases belong to the variety sometimes called nephritis repens or reticular nephritis In this condition a very chronic inflammatory process creeps through the kidney giving rise to patchy destruction and fibrosis of the parenchyma In childhood and early adult life there is not much arteriosclerotic change but in the nephritis repens of middle age this may become so marked as eventually to cause cardiac failure The kidney varies in appearance according to the duration of the disease It is sometimes much reduced in size, white or

reddish in colour, scarred, coarsely granular or irregularly lobulated The cortex is much reduced and the distinction between cortex and medulla is blurred In other cases the organ is normal or nearly normal in size with fine granulations or none at all and with good demarcation between medulla and cortex Microscopically the affected areas show greater or less chronic inflammatory change with fibrosis, cellular infiltration and vascular thickening In the intervening portions there may be evidence of glomerular hypertrophy and tubular degeneration Lipoid deposits are usually seen

The characters of non-hæmorrhagic degenerative Bright's disease (the nephrosis of Volhard and Fahr) are marked albuminuria, low plasma albumin and consequently œdema which is often massive in character, raised plasma content of fat and cholesterol and absence of hæmaturia and hypertension The ability to excrete urea is usually said to be unimpaired but Van Slyke *et al* (*loc cit*) found a gradual diminution in the urea clearance in definite cases of nephrosis When this occurs the differentiation of pure degenerative nephritis from the chronic active stage of hæmorrhagic nephritis during a 'non-hæmorrhagic' period may be difficult The prognosis is different in the two conditions In the latter the usual ending is in uræmia In the former uræmia may also occur but some cases are said to end in recovery Others die from intercurrent streptococcal or pneumococcal infection Nephrosis appears to follow or accompany certain intoxications or infections among which are pregnancy, osteomyelitis, syphilis and tuberculosis but in many cases there is no obvious cause, the condition beginning insidiously without illness of any kind It is sometimes associated with amyloidosis Pure lipoid nephrosis is apparently a rare condition The kidneys are enlarged white in colour and of normal consistency The cortex is wide and pale and sharply separated off from the medulla Microscopically the outstanding change is marked lipoid or 'myelin' degeneration of the tubules, especially the convoluted tubules The vessels and glomeruli are usually normal but in old-standing cases some tufts may show hyaline changes and there may be proliferation of the parietal cells

In nephrosclerosis the essential lesions are vascular, the chief clinical features being a raised blood-pressure and cardiac hypertrophy with slight proteinuria Volhard and Fahr (*loc cit*) recognized two types, the benign and the malignant In the former the symptoms throughout the disease are attributable to circulatory changes in the kidneys and elsewhere and death is usually the result of heart failure rather than kidney failure Œdema is not present until the end and is then of cardiac origin, there being no reduction of the plasma proteins In the malignant form there is at some stage a rapid decrease in renal function and death is generally due to renal failure alone or to renal and cardiac failure combined In both forms the urea clearance is diminished owing to the lessened blood flow in the kidneys Red cells may be found in the urine but are often absent till the terminal stage In the pure arteriosclerotic form the changes are intimal hyperplasia of the large

and medium sized arteries with fatty degeneration and hyaline necrosis of the arterioles In the malignant form there are inflammatory changes as well 'such as necrosis and proliferations of the arterioli and glomeruli' (Van Slyke *et al*, *loc cit*) The vascular lesions lead to collapse and ischæmic atrophy of the glomeruli and tubules which eventually became hyaline scars There is marked fibrosis of the interstitial tissue which, by pressing on tubules still functioning, often leads to the formation of retention cysts of various sizes Macroscopically the kidneys are typically of the 'small red' variety

The patients on whom our observations were made sought admission to hospital chiefly on account of œdema and general weakness The main features are summarized in the appendix Histories were often difficult to obtain but most patients owned to having suffered from 'fever' and some from dysentery and enteric Enlargement of the spleen alone or of the spleen and liver was present in five, one of whom was found to be a case of chronic lymphocytic leukæmia With the possible exception of No 23, none gave a typical history of an acute attack of nephritis, although the 'fever' which was said by some to be the starting point of the œdema may have been due to acute renal involvement or to an acute exacerbation of a chronic nephritis In the majority, however, it is more than likely that the dropsy developed gradually without pyrexia or other marked symptom Five died in hospital, the essential post-mortem details are given in the summary of cases

Of the twenty-five patients examined, six (Nos 9, 10, 11, 12, 13 and 21) showed pronounced cardiovascular changes, viz, increased blood-pressure with vascular thickening and left ventricular hypertrophy Circulatory changes would seem to have been primary in Nos 9, 10 and 12, although the cause of death in Nos 9 and 10 was more renal than cardiac and No 12 was almost in uræmia when he left hospital The urea clearance was under 10 per cent of normal in all three cases In Nos 9 and 10 the kidneys were of the 'small red' variety and microscopic examination showed not only generalized fibrosis, arterial and arteriolar thickening and hyaline occlusion of the glomeruli and capillaries but inflammatory changes as well We consider that these three cases were examples of the malignant sclerosis of Volhard and Fahr In No 11, in whom the efficiency of the kidney was still over 60 per cent of normal when he left hospital, the primary condition was also apparently vascular, the heart being more affected than the kidneys No 13 died in the terminal stage of hæmorrhagic nephritis, the cause of death being partly renal and partly cardiac The urea clearance was 9 4 per cent of normal The kidneys were 'small white' in type Microscopic examination revealed degenerative changes in the tubules with inflammatory and destructive changes in the glomeruli Thickening and obliteration of the blood vessels were not marked In the case of No 21, death occurred from lobar pneumonia when the urea clearance was still 50 per cent of normal No red blood cells were found

in the urine on several occasions The kidneys showed the changes associated with nephritis repens Microscopically areas of dense chronic inflammatory change alternated with areas in which the only departures from normal were some dilatation and 'fatty' degeneration of the tubules

In connection with the above cases it should be stated that in the terminal stages of hypertensive renal disease it may sometimes be very difficult during life to decide whether the vascular changes are of a secondary nature or are the primary cause of the disease unless a definite history is forthcoming The blood urea is high in both conditions Evidence of vascular disease elsewhere is an important deciding factor Van Slyke *et al* (*loc cit*) point out that it may also be difficult to say whether, in a terminal case of hæmorrhagic nephritis with arterio- and arteriolo-sclerosis, the latter condition originated independently of the former They describe two cases in whom they believed both types of nephropathy to have existed

The only case of the series which might be classified as primarily purely degenerative was No 6 The inflammatory changes found in the kidney post-mortem were acute in character and probably embolic in origin There was extensive amyloid degeneration of the glomeruli and blood vessels and lipoid degeneration of the tubules so that the condition was one of amyloid nephrosis The blood urea was about normal and the urea clearance 65 5 per cent of normal A few red cells were present in the urinary deposit, the result probably of the secondary acute changes The patient stated that the œdema began after an attack of pneumonia but the amyloidosis was undoubtedly connected with the chronic tubercular disease found in both lungs The Wassermann reaction was negative

In all the remaining cases the findings indicated hæmorrhagic nephritis with different degrees of renal insufficiency No 8 died of uræmia when the urea clearance had fallen to 5 per cent of normal Proteinuria was 2 2 per cent Post-mortem changes indicated a sudden change from the chronic active to the terminal stage, the disease being of fairly recent origin Although the kidneys were enlarged and 'chronic parenchymatous' in appearance there was extensive glomerular destruction Vascular changes were slight Nos 1, 2 and 19 had renal clearance values below 20 per cent of normal and were therefore in the terminal stage Nos 2, 16 and 20 were sufficiently long under observation to ascertain the general trend of the disease and in Nos 2 and 16 at least this was steadily downward No 20 showed no diminution in the urea clearance after six months but the blood-pressure had risen 10 mm Hg No 3 is worthy of special mention as the characteristic features of chronic lymphocytic leukæmia were present and the Wassermann reaction was strongly positive Ocular symptoms were not present in any patient

It will be noticed that in all cases the œdema, on account of which the patients came to hospital, had been present for a short time only, viz , $1\frac{1}{2}$ to 6 months and

that except in one (No 4) there was no mention of a previous attack If we regard the kidney disease as having existed only during the period of the œdema most cases would be classified as acute and two, Nos 12 and 19, would appear to have passed directly from the acute to the terminal stage, as the change from the acute to the chronic stage usually occurs at 4 months (Van Slyke *et al*, 1930) In No 13, however, 'small white' kidneys were found at autopsy, indicating disease of long standing The post-mortem appearance in No 21 also pointed to a very chronic process which in this instance had been cut short by death from lobar pneumonia, and even in No 8 who had 'large white' kidneys the microscopic appearances showed that the renal disease must have been going on for some considerable time There is reason for thinking, therefore, that in at least some of the patients who did not come to autopsy one was also dealing with an exacerbation of a chronic latent condition rather than with an initial attack of nephritis In the type of patient we were concerned with if the disease began in an insidious manner and gradually passed into a latent form, (nephritis repens in fact), it might easily be overlooked or neglected until the onset of intercurrent illness, malnutrition or possibly some other factor caused it to assume an acute or chronic active form or hastened it on to the terminal stage

Our investigations throw no new light on the cause of hæmorrhagic nephritis This condition is generally supposed to be related to streptococcal infection and to be allergic in nature The association of rheumatic joint pains in two cases (Nos 1 and 2) with the onset of renal œdema is of interest in this connection We found no evidence of any direct relationship between the renal condition and the malaria which probably all our patients had suffered from Malaria, like enteric fever, dysentery or other diseases, may of course be an aggravating factor It should be stated that Giglioli (1930) considers that malaria, especially the tertian and quartan forms, is the cause of endemic nephritis in British Guiana

We would emphasize the value of the urea clearance test as a measure of renal efficiency Our cases, though few in number, bear out the claims made on its behalf It has, however, one drawback in certain instances Results are reliable only if the total amount of urine secreted during the period of the test is obtained Some patients may not, for some reason or other, empty the bladder completely and if this happens the result of the test will be too low Catheterization may be resorted to but is not always very satisfactory It is possible, theoretically at least, to get over the difficulty by so regulating the intake of fluid that the urinary output measures about 1 c c per minute The clearance is then $\frac{U}{B}$

In the matter of treatment we have only to mention the fact that following Peters *et al* (1926), we allowed cases of œdematous hæmorrhagic nephritis a liberal

supply of protein in the diet with good results The normal diet of many of our patients contains only a small amount of protein

SUMMARY

Twenty-five cases of œdematous nephritis were investigated from the point of view of the nature of the disease process Renal efficiency was determined by the urea clearance test

Four cases were considered to have been of the nephrosclerotic type Two of these died from uræmia, when the urea clearance was 7 9 per cent of normal and 3 5 per cent of normal respectively The pathological changes in both were indicative of the 'malignant sclerosis of Volhard and Fahr

In one individual the lesions were those of amyloid nephrosis Death occurred from secondary broncho-pneumonia when the urea clearance was 64 9 per cent of normal

The remaining twenty cases were all of the 'hæmorrhagic' variety (glomerulo-nephritis) One died from uræmia when the urea clearance had fallen to 5 1 per cent of normal The post-mortem changes indicated that a sudden transition to the terminal stage had occurred fairly early in the course of the disease In another death occurred from renal and cardiac failure when the urea clearance was 9 4 per cent of normal The kidneys were 'small white' in type and there was marked hypertrophy of the left ventricle A third patient died of pneumonia when the renal function was still 60 per cent of normal The kidney lesions were typical of nephritis repens Reasons are given for considering that in at least some of the surviving patients the disease was of a prolonged latent type which passed into an active stage shortly before admission to hospital

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APPENDIX

SUMMARY OF CASES

1 *22nd November*, 1930 A R, 28, Muslim male Marked generalized œdema of two months' duration Began with 'fever' and painful swollen joints (knees and ankles) History of malaria and dysentery Urine reduced in amount Urinary deposit many red cells, leucocytes, granular and epithelial casts Urea clearance 7 5 per cent of normal Blood urea 102 5 Proteinuria large amount Urea concentration test, 2 17, 1 5, 1 6 Blood pressure 115 75 Heart apparently normal Leucocytes 9,600 Wassermann reaction negative

2 *10th December*, 1930 S S, 40, Muslim male Fairly marked generalized œdema with painful knee, ankle and elbow joints Joints not swollen Œdema of feet came on 4 months ago after the joints had become painful The face became swollen only a fortnight ago Urinary deposit many red cells, a few leucocytes, granular, epithelial and fatty casts Urea clearance 21 4 per cent of normal Blood urea 87 7 Proteinuria marked Urea concentration test 0 5, 0 6, 0 7 Blood pressure 130 90 Heart apparently normal

10th March, 1931 Red blood cells only 2 millions Leucocytes 7,000 General condition somewhat improved subjectively Urea clearance 12 5 per cent of normal Blood urea 64 7 Proteinuria 1 per cent Blood pressure 120 90 Wassermann reaction positive Plasma cholesterol 200 mg per 100 c cs

3 *15th January*, 1931 N D, 50, Muslim male Marked generalized œdema and weakness Œdema came on insidiously a month ago Liver and spleen enlarged Very anæmic (Hb 30 per cent red cells 1¾ millions), white cells 288 000 Diff count —lymphocytes 94 per cent, polynuclears 3 per cent, monocytes 2 per cent, eosinophiles 1 per cent (Chronic lymphocytic leukæmia) Enlargement of axillary and inguinal glands Wassermann reaction positive Urea clearance 21 4 per cent

of normal Blood urea 56 7 Proteinuria 0 7 per cent Urinary deposit many red and white cells, hyaline granular and epithelial casts Blood pressure 135 75 Heart slightly enlarged to the left

4 *7th February* 1931 M A, 10 Muslim male Marked *generalized œdema* Apparently œdema first occurred 3 years with fever but gradually disappeared About a week ago it reoccurred *in its* present form also with fever History of malaria Spleen 3 inches below costal margin Liver slightly enlarged White cells 6 000 Urine diminished and somewhat smoky in colour Urinary deposit many red cells, a few white cells, many granular fatty and hyaline casts Urea clearance 51 9 per cent of normal Blood urea 31 5 Proteinuria 0 8 per cent Blood pressure 110 75 Heart apparently normal Wassermann reaction negative

5 *11th February* 1931 H, 50, Muslim male Marked generalized œdema which developed after an attack of 'fever' lasting a month about three months ago History of malaria and chronic bronchitis White cells 10 000 Urine about 18 oz in 24 hours Urinary deposit some red cells and granular fatty and hyaline casts Urea clearance 26 2 per cent of normal Blood urea 32 7 Proteinuria 0 9 per cent Blood pressure 97-70 Heart apparently normal Wassermann reaction negative

9th March, 1931 Œdema less Urine increased in amount Urea clearance 33 3 per cent of normal Blood urea 22 2 Proteinuria 1 1 per cent Plasma cholesterol 256

6 *7th February*, 1931 M S 35, Sikh male Very pronounced generalized œdema of one month's duration Apparently came on after an attack of pneumonia History of typhoid (?), malaria and syphilis but Wassermann reaction was negative Bronchitic signs on admission White cells 7,000 Urine diminished Urinary deposit few red cells, many granular fatty, epithelial and hyaline casts Urea clearance 61 9 per cent of normal Blood urea 23 0 Proteinuria 1 8 per cent Blood pressure 115 80 Heart apparently normal

Patient died after 18 days in hospital from broncho pneumonia A condition of old fibroid phthisis in both lungs was also found The kidneys were enlarged, pale and soft Capsule not adherent Marked distinction between medulla and cortex, the latter being increased in width and pale in colour Some injection of the pelvic mucous membrane On microscopic examination the chief features were degeneration of the tubules especially the convoluted tubules, some glomeruli almost normal, others hyaline in appearance in different degrees, hyaline streaks in tubules and vessels Special staining showed this hyaline material to be amyloid There were acute inflammatory areas in the cortex apparently of recent development and characterized by marked polynuclear infiltration They were probably of embolic origin

7 *18th February*, 1931 C D, 40, Muslim male Marked generalized œdema of two months' duration following on a week's 'fever' History of malaria and dysentery Spleen slightly enlarged Urine diminished in quantity Urinary deposit few red and white cells, epithelial, granular, fatty and hyaline casts Urea clearance 27 7 per cent of normal Blood urea 44 5 Proteinuria 1 4 per cent Blood pressure 135 90 Heart apparently normal

8 *2nd March*, 1931 S M, 20, Muslim male Marked generalized œdema of 3 months' duration Apparently came on insidiously but there was a history of a week's 'fever' two months previously History of malaria *Urine much diminished in quantity Urinary deposit many* red cells, a few white cells, granular, epithelial and hyaline casts Urea clearance 5 1 per cent of normal Blood urea 89 2 Proteinuria 2 2 per cent Blood pressure 160 110 Heart not enlarged Wassermann reaction negative Plasma cholesterol 303 Red cells 2 6 millions Leucocytes 4,500 Many hook worm eggs in stools

Patient died after 21 days in hospital from uræmia Only kidneys could be examined They were found to be enlarged, mottled and soft Capsule not adherent Cortex wide and pale Microscopically they showed degeneration of the tubules, especially the convoluted tubules, increased nuclei in some glomerular tufts, hyalinization in others and occasionally thickening of the glomerular capsule The picture was one of hæmorrhagic nephritis with pronounced glomerular changes

9 *26th March*, 1931 H R, 40, Hindu male Marked ascites and œdema of legs, breathlessness and palpitation Ascites was of four months' duration Swelling of the legs on exertion Heart

enlarged downward and to the left No murmurs Wassermann reaction negative Hookworm ova present in small numbers Spleen somewhat enlarged Red cells 4 8 millions Leucocytes 10,000 Has had no previous illness Urine rather increased in amount Urinary deposit a few red cells, a few leucocytes and some hyaline and granular casts Urea clearance 7 9 per cent of normal Blood urea 119 7 Proteinuria 0 2 per cent Blood pressure 160 105

Patient died after about a month in hospital from renal failure Post mortem examination showed emphysematous lungs, enlarged malarial spleen with perisplenitis, early atrophic cirrhosis of the liver, much enlarged left ventricle with mural endocarditis and small red granular kidneys The renal capsule was adherent in places and the cortex irregularly reduced in width and mottled There were several cysts on the surface The medulla and cortex were not sharply demarcated Microscopically the kidneys were very fibrous, many glomerular tufts being represented by fibrous masses There was much thickening of Bowman's capsule in many glomeruli Some tufts that were not fibrous showed great increase in nuclei The arterial and arteriolar walls were much thickened The tubules were dilated in places

10 *27th March*, 1931 S N , 30, Hindu male Swelling of the lower limbs and puffiness of the face of one month's duration, palpitation of the heart and breathlessness No history of other illness The heart was enlarged downwards and to the left No murmurs Wassermann reaction negative Urine normal in amount Urinary deposit a few red cells, hyaline and granular casts Urea clearance 3 5 per cent of normal Blood urea 401 5 Proteinuria 0 1 per cent Blood pressure 170 125 Plasma cholesterol 195

Patient died from uræmia after about 5 weeks in hospital, 10 days after the above examination Post mortem examination showed a very much hypertrophied left ventricle, the ventricular wall being about 8 times the normal The kidneys were small, with slightly adherent capsules The surface was uniformly granular and was of a mottled red and grey colour The same mottling was seen on section, especially in the medulla There was much fat in the pelvis Microscopic examination showed marked thickening of the arteries and arterioles, some fibrosis and increase of nuclei in the glomerular tufts, general increase of fibrous tissue and dilation of some tubules

11 *1st May*, 1931 B R , 47 Hindu male Marked generalized œdema of 3 months' duration, first noticed on the face Palpitation of heart for 3 months also History of chronic bronchitis but of no other illness Liver enlarged four fingers below costal margin Urine diminished in amount Urinary deposit an occasional red and white cell, granular, hyaline and epithelial casts Urea clearance 56 2 per cent of normal Blood urea 35 0 Proteinuria small amount Blood pressure 170 140 Both right and left sides of the heart enlarged No murmurs Hypertrophy of the left ventricle Wassermann reaction negative Leucocytes 5,500

12 *2nd May*, 1931 M R , 45, Hindu male Œdema of legs and ascites of 4 months' duration Frequency of micturition for the past year History of malaria and enteric (?) Spleen 2 inches below costal margin Left ventricular hypertrophy No murmurs Polyuria Urinary deposit occasional red cells, hyaline and granular casts Proteinuria 0 05 per cent Urea clearance 4 6 per cent of normal Blood urea 179 0 Blood pressure 170 110

13 *8th May*, 1931 A B , 40, Muslim male Pronounced generalized œdema of six months' duration Onset insidious History of no acute illness other than intestinal colic Breathless and cyanosed Left ventricular hypertrophy Right cardiac enlargement Urine diminished in quantity Urinary deposit red cells, white cells, granular and hyaline casts Urea clearance 9 4 per cent of normal Blood urea 240 0 Proteinuria 0 4 per cent Blood pressure 180 120 Wassermann reaction negative Leucocytes 7,000

Patient died four days after admission on 9th May, 1931 Post mortem examination showed the immediate cause of death to be acute pulmonary œdema There was marked left ventricular hypertrophy No murmurs Right heart dilated The kidneys were small, capsule just adherent, surface finely granular On section the appearance was that of 'small white kidney' Microscopically there was generalized fibrosis Some glomeruli were almost completely hyaline, others showed

increase in the nuclei of the tufts and others were almost normal Vessels not markedly thickened Tubules showed degenerative changes

14 *3rd June*, 1931 V R, 14, Muslim female Marked generalized œdema of two months' duration Onset insidious History of irregular fever Had two bouts of temperature (to 101°F) while in hospital (? malaria) Blood pressure 110 80 Heart apparently normal Urine diminished in quantity Urinary deposit many red cells, some white cells, granular, hyaline and epithelial casts Urea clearance 49 3 per cent of normal Blood urea 21 5 Proteinuria 0 15 per cent Wassermann reaction negative Leucocytes 6,250

15 *9th June*, 1931 H C, 15 Hindu male Pronounced generalized œdema of one month's duration Onset insidious Occasional bouts of low fever 99°F or so Heart apparently normal Blood pressure 118 80 Urine diminished in quantity Urinary deposit red cells, a few white cells, granular and epithelial casts Urea clearance 57 5 per cent of normal Blood urea 39 5 Proteinuria 2 1 per cent Wassermann reaction negative

16 *28th October* 1931 R, 40, Muslim male Fairly marked generalized œdema of three months' duration Onset apparently insidious Occasional rises of temperature (? malaria) Heart apparently normal Blood pressure 112 70 Urine much diminished in amount Urinary deposit many red cells, some white cells, granular, epithelial and hyaline casts Urea clearance 37 1 per cent of normal Blood urea 35 0 Proteinuria 0 6 per cent Wassermann reaction negative Urea concentration test (*on 7th November, 1931*) 1 2, 2 0, 2 2

14th December, 1931 Œdema somewhat less No fever Blood pressure 128 104 Urine increased in amount Urinary deposit as before Urea clearance 26 6 per cent Blood urea 51 0 Proteinuria 1 8 per cent Plasma proteins total 4 92 per cent, albumin 1 2 per cent, globulin 2 9 per cent, fibrinogen 0 82 per cent

17 *3rd November*, 1931 J N, 31, Hindu male Pronounced generalized œdema of 5 months' duration States that it came on gradually after a febrile illness lasting 15 days This illness ended with the appearance of a rash all over the body (? dengue) Has had occasional short attacks of 'fever' Blood pressure 128 80 Heart apparently normal Urine much diminished in amount Urinary deposit red cells and an occasional leucocyte, some granular, epithelial and hyaline casts Urea clearance 73 per cent of normal Blood urea 34 5 Urea concentration test 2 9, 3 0, 2 7 Proteinuria 0 3 per cent

18 *4th January*, 1932 H A, 18, Muslim male Fairly marked generalized œdema which developed after an attack of lobar pneumonia about a month ago History of occasional attacks of 'fever' Blood pressure 120 80 Heart apparently normal Wassermann reaction negative Urine diminished in amount Urinary deposit many red cells, few leucocytes, hyaline, fatty and granular casts Urea clearance 63 6 per cent of normal Blood urea 18 5 Proteinuria 1 1 per cent

19 *19th January*, 1932 K M, 35, Muslim male Œdema of lower limbs of 1½ months' duration Developed after a febrile illness lasting twenty days History of syphilis 22 years ago Wassermann reaction positive Subject to short bouts of 'fever' (1 3 days) Blood pressure 120 82 Heart apparently normal Urine diminished in amount Urinary deposit some red blood cells, leucocytes, epithelial and granular casts Urea clearance 10 7 per cent of normal Blood urea 85 0 Urea concentration test 1 1, 1 6, 1 6 Proteinuria 0 6 per cent Plasma proteins total 6 08 per cent, albumin 1 90 per cent, globulin 3 67 per cent, fibrinogen 0 51 per cent Hæmoglobin 68 8 per cent Leucocytes 11,000

20 *15th October*, 1931 D, 26, Hindu male Œdema of lower limbs and ascites of 2 months' duration Seems to have had a fairly sudden onset Some dyspnœa Liver slightly enlarged History of dysentery No entamœbic cysts found Has occasional rises of temperature (? malaria) Blood pressure 115 95 Heart apparently normal Wassermann reaction negative Urinary deposit some red cells and leucocytes, granular, epithelial and hyaline casts Urea clearance 48 per cent of normal Blood urea 46 0 Proteinuria 0 65 per cent Urea concentration test on *22nd* October, 1931, 2 6, 3 2, 2 8

9th March, 1932 Puffiness of the face, slight œdema of the legs and ascites Fluid in the pleural cavity Blood pressure 125 105 Heart not enlarged Urinary deposit an occasional red and white cell, granular and hyaline casts Urea clearance 54 2 per cent of normal Blood urea 50 0 Proteinuria 0 75 per cent

21 *12th November*, 1931 H D, 40, Hindu male Ascites and œdema of lower limbs of 3 months' duration Liver 1½ inches below costal margin Spleen just palpable History of vague 'fever' in early life Says he had syphilis but Wassermann reaction negative Blood pressure 198 110 Marked hypertrophy of left ventricle No murmurs Urine increased No hæmaturia, hyaline and granular casts Urea clearance 59 2 per cent of normal Blood urea 34 4 Proteinuria 0 1 per cent

Patient died of lobar pneumonia after 6 weeks in hospital Post mortem examination revealed in addition to pneumonic consolidation of the lower part of the left lung, a very much enlarged heart There was marked hypertrophy of the left ventricle and some hypertrophy of the right No valvular disease The liver was nutmeg The kidneys were small, red in colour with adherent capsule and lobulated granular surface Microscopic examination showed areas of chronic inflammatory change surrounded by tissue which was almost normal In the former there was marked round cell infiltration of the glomeruli with thickening of Bowman's capsule and in some places adhesion between the tuft and the capsule The interstitial tissue was increased and the tubules more or less degenerated In the non inflammatory areas there was some 'fatty' degeneration of the epithelium of the convoluted tubules and occasional enlargement of the glomeruli which otherwise seemed normal

22 *23rd February*, 1932 F, 22, Hindu male Marked generalized œdema of two months' duration, which is stated to have followed a period of irregular malaria like fever lasting 1½ months Blood pressure 90 60 Heart apparently normal Urine diminished in amount Urinary deposit red blood cells, leucocytes, hyaline, epithelial and granular casts Urea clearance 40 8 per cent of normal Blood urea 29 5 Proteinuria 0 4 per cent Wassermann reaction negative

23 *26th February*, 1932 M, 30, Hindu male Very pronounced generalized œdema of two months' duration, which is stated to have followed a febrile illness lasting three months The urine was 'red' in colour during this illness (? pigment) History of renal colic Febrile on admission (102°F) for one day only Spleen and liver slightly enlarged Heart apparently normal Blood pressure 155 95 Urine diminished in amount Urinary deposit many red cells, some white cells, granular, fatty and epithelial casts Urea clearance 18 6 per cent of normal Blood urea 97 0 Proteinuria 0 3 per cent Wassermann reaction negative

24 *1st March*, 1932 A de S, 43, Christian male Generalized œdema of one month's duration The œdema came on fairly suddenly without any 'fever' or other illness Heart apparently normal Blood pressure 160 110 Slight enlargement of the heart Urine much diminished amount Urinary deposit red blood cells, a few leucocytes, hyaline, granular and fatty casts Urea clearance 41 3 per cent of normal Blood urea 94 0 Proteinuria 0 9 per cent Wassermann reaction negative

25 *2nd March*, 1932 D S, 24, Hindu male Marked generalized œdema of two months' duration Followed a febrile illness lasting five days during which there was vomiting and diarrhœa Blood pressure 130 90 Heart apparently normal Urine diminished in amount Urinary deposit some red blood cells, granular, fatty, epithelial and hyaline casts Urea clearance 37 6 per cent of normal Blood urea 28 5 Proteinuria 0 75 per cent Wassermann reaction negative

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THE CLIMATE IN WHICH THE RAT-FLEA LIVES

BY

P A BUXTON,

(*From the Department of Entomology, London School of Hygiene and Tropical Medicine*)

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I THE PROBLEM

IT is a general truth that epidemics of plague come to an end when the weather is hot and dry, and that the commencement of an epidemic and its continuance are favoured by certain conditions of temperature and humidity It is also known that it is the seasonal abundance of rat-fleas, particularly of *Xenopsylla cheopis*, which determines the outbreak of epidemics of plague among rats and man and it is the larva of the flea and not the adult which is most susceptible to unfavourable conditions (Mellanby, 1932) It appears also that the geographical distribution of plague is largely, but not entirely, determined by climatic conditions which are favourable to this species of flea

But though there are grounds for relating numbers of fleas, and epidemics of the disease, to climatic factors, we still employ ordinary meteorological data in our studies Even when the data are complete and based on readings taken throughout the 24 hours, one would suppose that there is no relation between the climate in the rat's nest and in the standard Stevenson's screen frequently the only data are those taken once (or twice) during the day We cannot criticize the meteorologist for the way in which his data are collected and presented, standard methods are necessary for his purpose and we are grateful for the great mass of fact which he has accumulated But the biologist must take the blame for having so little knowledge

of the climate which prevails where the insects normally live We need readings, preferably taken with recording apparatus, from within warehouses, also from rat holes and similar places The essential elements of climate appear to be temperature and humidity

With regard to the temperature and humidity in soil, we have a little knowledge

Temperature—This subject has been recently summarized by Keen, he discusses soil temperature in relation to latitude, altitude, vegetation, nature of the soil season, and many other factors He also deals with the theory of the flow of heat through a mass of soil To his references, one may add papers by Kashkarov and Kuibatov McKenzie-Taylor and Burns (1924*a* and *b*), Sinclair, and Williams (1923, 1924*a* and *b*) It must be realized that, even a few inches below the surface of the soil, the temperature is very nearly stable between day and night This is true even in the extreme climate of a desert, where the daily and seasonal fluctuations of temperature in the air are so great For instance, Williams (1923, 1924*a* and *b*) has shown that the high maximum at midday was confined to the surface of the soil the range of temperature and the daily maximum *fall rapidly at 10 or 20 cm* depth in the sand At 20 cm below the surface, the mean daily range of temperature is about 6 0°C in summer, and half that in winter, the mean temperature in summer is about 33°C in winter 17°C to 20°C If, therefore, an animal can burrow to this depth, it will find itself in very equable conditions, at any time of year, so long as it remains in its hole Uvarov suggests that bubonic plague survives in the steppes surrounding the Caspian Sea because the fleas in the burrows of the ground squirrel, *Spermophilus*, are protected against the cold of winter and the dry heat of summer

Humidity—Our knowledge of temperature beneath the surface of the ground is not inadequate, and methods of measuring and recording it exist With regard to humidity in rat holes, etc, I can find no information except that collected by Ingram He made an 'artificial burrow' which consisted of an animal cage buried 30 inches below the level of the ground In it the temperature ranged from 78 8°F to 74 0°F (26 0°C to 23 3°C), the range in a screen was 92 5°F to 58 6°F (33 6°C to 14 8°C) that is to say, the range in the burrow was a seventh of that in the screen The saturation deficiency in the burrow measured twice daily, was about 6 3 mm (0 27 in) of mercury that in the screen averaged 11 mm (0 437 in) at 7-30 A M, and 27 mm (1 093 in) at 2-30 P M The measurements of humidity may be criticized on technical grounds, but the figures certainly show that the conditions below the soil were stable and damp (*see also* Hirst, for sewers in Colombo, Ceylon)

Much more work of this sort is needed, and the present paper describes an attempt to increase knowledge The technical problems of hygrometry in places which are small and inaccessible are still difficult in particular we lack apparatus which will give a current record of changes of humidity An additional difficulty

is that there are several scales of humidity in use It is essential to grasp the difference between them, some people have been helped by a diagram published elsewhere (Buxton, 1930 fig 1) One must also know which scale is appropriate for a given purpose, and become familiar with methods of converting readings from one scale to another, this is not possible unless the temperature is known

The three principal scales of humidity are as follows —

1 *Absolute humidity* —This gives the total amount of water in a unit volume of air It may be expressed as milligrams per litre, but the most convenient notation is to express it as a vapour pressure in millimetres or inches of mercury In ordinary practice the absolute humidity is obtained from the readings of dry and wet bulb, by means of tables The Indian tables (*see* Simpson) cover nearly all conditions which one is likely to encounter The absolute humidity is useful if one wishes to study movements of water vapour in space for instance, water vapour will diffuse out of a cave into the open air if the absolute humidity is higher in the cave

2 *Relative humidity* —This is the amount of water vapour in a space compared to what the space could hold if it were saturated at the same temperature The proportion is generally expressed as a percentage For instance, if a room holds three-quarters of the water vapour which it would hold if saturated at the same temperature, it is said to have a relative humidity of 75 per cent The degrees of relative humidity which correspond to particular wet and dry bulb temperatures may be obtained from the Indian tables Relative humidity is important partly because most meteorological tables are constructed on this scale, partly because the hair hygrometer and similar instruments record the relative humidity irrespective of temperature

3 *Saturation deficiency* —This is the amount of water vapour which would have to be added to that already there in order to saturate a given space, temperature remaining constant The saturation deficiency is always expressed in absolute units (generally mm of mercury) it is obtained by subtracting the absolute humidity from the saturation vapour pressure (i e , the total amount of water which air would contain if it were saturated at the same temperature) At first sight it appears as if it were the difference between the relative humidity and saturation e g , if the relative humidity is 75 per cent, one is inclined to say that the saturation deficiency is 25 per cent (i e , 100—75) But this is a fallacy, because the total amount of water vapour required to saturate a space is greater, the higher the temperature Therefore the amount of additional water vapour required to bring air from 75 per cent to saturation is greater, the higher the temperature The insect physiologist must become familiar with this measure of humidity, for it was shown by Bacot and Martin that at 32°C and presumably at other temperatures the duration of life of the adult *Xenopsylla cheopis* is proportional to the saturation deficiency of the atmosphere I have recently shown (Buxton, 1931*b*) that this law

is of very wide application in insect physiology, but we cannot yet say that it applies to all insects or that it is operative under all conditions of temperature and humidity It will also be remembered that Brooks and also Rogers have been able to relate many events in the epidemiology of plague in India to saturation deficiency we may assume that this effect of climate is upon the fleas and their larvæ

In the present paper, the facts relating to humidity are sometimes referred to one scale, at others to another I have done this deliberately, because one cannot think adequately about the ecology of plague unless one is prepared to use each of the three scales for different purposes each scale is appropriate

II The methods

Knowledge of the climate of the places where fleas actually live might be extended in three stages We might study the climate in grain stores, cellars, etc , secondly we might investigate the conditions in the rats' holes or among sacks of corn , thirdly, pursuing the matter to its logical end we might study the climate among the rat's hairs, in its nest, or in the dust in which the larvæ are found In this paper I discuss temperature and humidity in stores and cellars, also in rat holes , the third stage is still untouched The data here given were accumulated in Jerusalem and Haifa, Palestine, in June and July 1931

Stores and cellars —The standard methods of meteorology are applicable to the study of climate in a grain store or cellar I used maximum and minimum thermometers, also thermohygrographs which were checked twice daily by a whirling psychrometer, the readings of which were reduced by the well-known tables of the Prussian Meteorological Institute (Hellmann) In both the places in which I worked (Haifa and Jerusalem), I was able to expose duplicate instruments in the Stevenson's screens of the Department of Agriculture This gives a direct comparison between the climate of the store or cellar, and that of the external atmosphere

Rat holes and other small spaces —There is no great difficulty in studying the temperature 2 or 3 feet down a rat hole I tied thermometers about 4 inches long to pieces of wire and pushed them down the hole , as one may assume that the temperature varies a few degrees between day and night, this method is not unsatisfactory More complete results could be obtained with a distance thermograph, but none was available

The study of humidity is much more difficult Wet and dry bulb thermometers are unsuitable, partly because they cannot be ventilated, and partly because evaporation from the wet bulb will raise the humidity in the small space which is under investigation No standard method is applicable, and no ordinary instrument can be used One must work on small samples, and remove them from the rat hole with an appropriate apparatus I found it best to suck small quantities

of air into a dewpoint apparatus, such as is shown in Fig 1 The results obtained are made use of in the following way The thimble is chilled by evaporating ether inside it, and the first deposit of dew is observed when the thermometer in the thimble reaches (say) 20°C , as the temperature is allowed to rise the film is observed to disappear at the same temperature This shows that the amount of water vapour in the sample of air is enough to saturate it at 20°C , but reference to

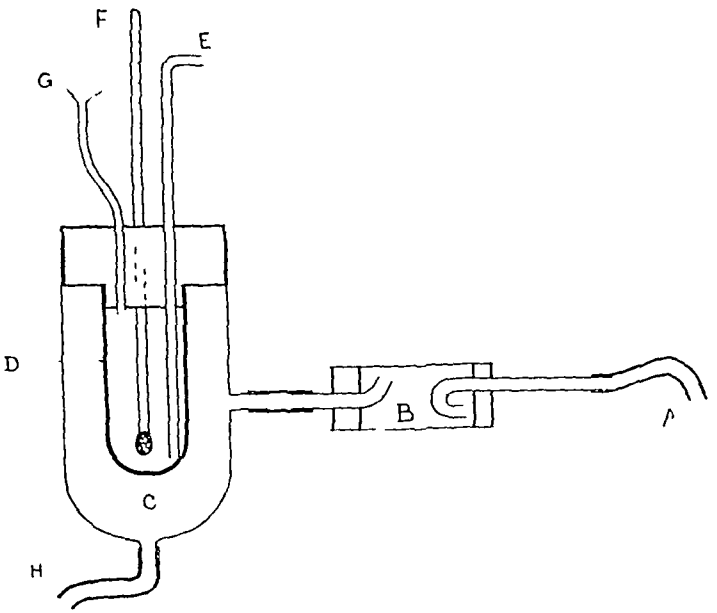

Fig 1 —Apparatus for determining the dewpoint of a sample of air, aspirated from within a rat hole, or from some other small and inaccessible situation The rubber tube A is pushed down the hole B is lined with vaseline and acts as a trap for dust The sample of air is studied inside C , it is sucked into the apparatus by mouth, through H D is the thimble on which the dew is observed it is chilled by evaporating ether inside it, by bubbling air with a small hand bellows attached to E Ether was previously introduced through G, and the air from E escapes through it F is the thermometer

physical tables (e g , Kaye and Laby, p 42) shows that saturated air at 20°C has a vapour pressure of 17 5 mm of mercury But the temperature in the rat hole was (perhaps) 27°C , and it would require a vapour pressure of 26 6 mm to saturate it , the saturation deficiency in the rat hole was therefore 26 6—17 5 = 9 1 mm of mercury If we desire the relative humidity, it is the absolute humidity observed, expressed as a percentage of the vapour pressure which would have produced saturation i e , $\frac{17\,5\times 100}{26\,6}$ = 65 8 per cent,

In Palestine where all walls and floors are masonry I found great difficulty in getting a rubber tube more than 2 feet or occasionally 3 feet down a hole It was much easier to manipulate a thick-walled than a thin-walled rubber tube In countries in which wood floors are used, one could certainly obtain valuable facts by drilling holes and sampling the atmosphere beneath the floor In using the dew-point apparatus (Fig 1), attention should be given to the following points If dust is sucked into the apparatus it becomes difficult to observe the dewpoint, the dust must not be filtered out with wool, or even with glass-wool as both these substances alter the humidity of the air which passes through them, but it can be trapped on a vaseline surface as shown in Fig 1 The use of a rubber tube is not wholly free from objection, as this substance is hygroscopic, but the rapid passage of a sample of air through the tube could hardly affect its humidity The principal objection to the method is that not all the air in the apparatus comes from the place which one wishes to study Some of the air, which was in the apparatus before the sample was taken, will still be there and may give a misleading result But if two or three samples are taken, till a consistent result is obtained, this objection is met Apart from this the apparatus is simple and portable, and in practised hands it is reliable

I have discussed elsewhere (Buxton, 1931*a*) other methods of hygrometry which might appear suitable The chemical hygrometer*, modified by Mellanby, is more accurate than the dewpoint apparatus But it is fragile and heavy, moreover it develops great errors if different parts of the apparatus are at different temperatures I therefore discarded it, after trial in the field but for laboratory studies it seems excellent I also attempted to use a small weighing hygrometer In rat holes and similar places it became dusty, and its weight was no longer an indication of the relative humidity of the atmospheres in which it had been exposed

III The results

In stores, barns and cellars

Fig 2 illustrates the type of difference recorded by thermohygrographs exposed in different places in the same town Three instruments were exposed, in a Stevenson's screen, in a small office in the port, and in a stable the places were all within a mile of one another and at sea-level, at Haifa The screen had a good meteorological exposure on a roof The office was on the first floor in a large Customs' shed, and its windows opened into the shed and not into the open air

* A number of improvements have been made since the instrument was figured in the paper quoted above The pumps designed to replace mercury reservoirs (Mellanby, 1931) enormously facilitate the work of analysis

It had a wood floor and ceiling It was used as a store for old papers, and was infested by rats The stable had a cobblestone floor, and solid mud walls It was infested with rats, and full of horses at night, when the doors and windows were shut The owner, his dog and chickens, also lived in it The floor was always moist with horses' urine and dung The instruments were exposed for the period 25th June to 4th July, and from the records 3-hourly means were calculated These

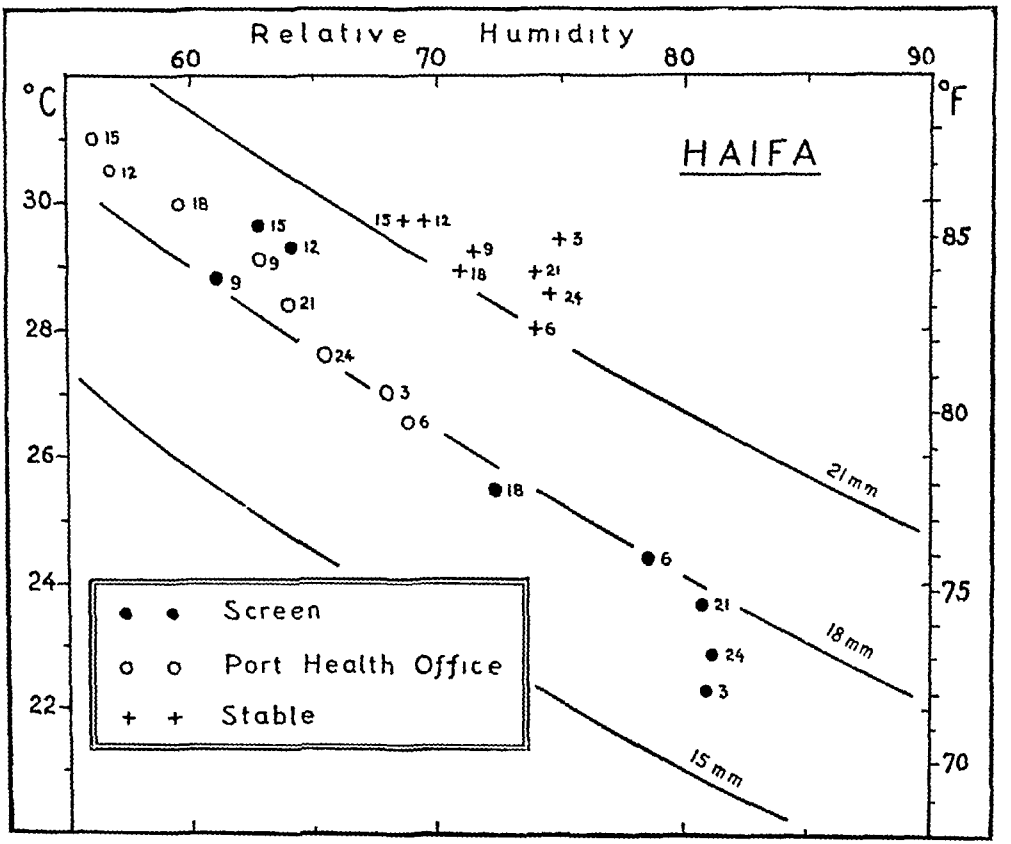

Fig 2—Three hourly means of temperature and relative humidity, derived from three thermohygrographs exposed in separate places in Haifa (25th June to 4th July) The three curves run through the points of which the water vapour pressures are 15, 18 and 21 mm of mercury

are shown on the graph, the figure '3' indicating the mean reading for 3 A M , over the above period , '12' is noon, and '24' midnight The records of humidity are made on the relative scale, and I have not converted them from this

The data shown in Fig 2 are very interesting The screen and the office differ in respect of temperature , at all times of the day and night the office is

hotter, the average* temperature of the office throughout the 24 hours is 28 8°C that of the screen is 25 8°C The absolute humidity in both is close to 18 mm of mercury, at all times of the day and night The differences in climate are therefore simple, depending entirely on temperature The climate in the stable is entirely different partly because it is shut up at night so that the range of temperature is much reduced But, owing to evaporation, the absolute humidity is 3 mm higher than that outside The differences between the climate here and outside are therefore complex

TABLE I

Three-hourly means, for 12 days (14th to 25th July) in Jerusalem, data derived from thermohygrographs exposed in a standard Stevenson's screen, also in a cowshed and the barn over it

Place	Time of day (hours)								Mean of 24 hours
	3	6	9	12	15	18	21	24	
Screen Temp °C	19 3	21 2	25 6	29 8	29 3	24 8	21 6	20 7	24 0
Shed „ „	24 2	24 0	28 3	28 5	28 0	26 7	25 5	24 7	26 2
Barn „ „	22 3	23 0	28 7	32 9	32 0	27 8	24 0	22 9	26 7
Screen Rel Hum per cent	74	71	55	44	48	67	73	75	63 4
Shed „ „	67	69	54	54	57	61	71	68	61 6
Barn „ „	71	69	48	37	40	53	70	65	56 6

Similar differences were observed in Jerusalem Thermohygrographs were exposed in a Stevenson's screen at the Directorate of Agriculture, also in a cowshed, and in the barn over the cowshed† The cowshed was well built, and kept dry, clean and ventilated, its climate was therefore not hot damp and equable like that of the stable at Haifa Three-hourly means of temperature and relative

* i e, the average of the 3 hourly means

† I am indebted to the Very Rev the Procurator of St Anne's Seminary, Jerusalem, for permission to expose instruments and pursue my studies in various parts of his farmyard

humidity are given in Table I Considerable differences will be noticed between the three places

I had not enough thermohygrographs to collect similar data elsewhere, but from time to time I made observations with a whirling psychrometer in a cellar in Haifa This cellar was beneath the building on the roof of which the Stevenson's screen stood a direct comparison of cellar and screen is therefore of interest The cellar had brick walls and a mud floor it was entered at one end by an open stair, at the other end there was a small chimney It was therefore well ventilated and one might have assumed that the absolute humidity of the air would be the same as that outside It hardly seemed likely that evaporation from the mud floor could raise the humidity, especially as only about an inch of rain had fallen during the previous four months at the time the observations were made (26th June to 3rd July) But in fact water was evaporating into the cellar, and affecting its climate The following figures are means, based on 11 observations, the vapour pressure in the cellar exceeded that in the screen eight times out of eleven, but the difference is not statistically significant owing to the scatter

Screen	Vapour pressure	14 8 to 21 2 mm,	mean	19 0 mm	(0 76 in)
Cellar	,, ,,	18 2 to 20 8 mm	,,	20 0 mm	(0 80 in)

Taking the individual readings in conjunction with the temperature, one finds that in the screen the saturation deficiency was 5 4 to 10 7 mm (mean 8 4 mm = 0 34 in), and in the cellar it was 3 2 to 5 0 mm (mean 3 7 mm = 0 15 in) The record is incomplete, because no readings were taken at night But this matters little, for during daytime, when the saturation deficiency is greatest, it was much less in the cellar than in the screen The cellar would therefore give an insect protection from dangerous loss of water at the most critical time in the 24 hours

In rat holes and other small places

The figures collected in rat holes and from within heaps of grain show great differences from one another and from those collected in the external atmosphere Table II gives some facts collected in St Anne's Seminary, Jerusalem The 'shed' was a well-built cowshed, with a stone floor and walls and a tile roof Readings were taken with a whirling psychrometer, whenever the climate in the rat holes was studied (by dewpoint) The two rat holes (A and B) went into the stone wall of the shed about 3 feet from the ground they were only a foot apart, but their climates were quite different for some unknown reason The reader will observe the great differences in humidity between the two holes, and the inconsistency of the data from day to day, especially in A It will also be noticed that the vapour pressure is nearly always higher in both holes than it is outside, showing that even in July evaporation was taking place in the holes Readings were only taken

during the daytime Let us assume that the climate in this shed was similar to that in the shed in Table I (which was of similar construction, and near at hand), then the night minimum temperature in the shed would have been 24°C, and the saturation deficiency about 8 mm The difference between shed and holes was therefore much greater by day than by night

TABLE II

Conditions in a shed in Jerusalem, and in two rat holes (A and B) which opened into it

Hour	SHED			HOLE A			HOLE B		
	Temp	Vap press	Sat def	Temp	Vap press	Sat def	Temp	Vap press	Sat def
9 0	29 2	13 6	16 8	27 0	26 0	0 6	27 0	17 0	9 6
8 30	28 6	11 4	18 0	27 0	25 2	1 4	27 0	23 1	3 5
8 30	27 0	14 7	11 8	27 0	21 6	5 0	27 0	14 5	12 1
8 30	25 6	15 4	9 2	27 0	23 7	2 9	27 0	14 5	12 1
17 0	27 8	15 6	12 2	27 0	16 5	10 1	27 0	15 5	11 1
9 0	26 0	15 5	9 6	27 0	18 6	8 0	27 0	15 5	11 1
Mean	**27·4**	**14·4**	**12 9**	**27·0**	**21·9**	**4·7**	**27·0**	**16 7**	**9·9**

Similar differences between conditions inside and outside rat holes are illustrated in Fig 3 On it I have plotted 17 readings taken in Haifa and Jerusalem during the hours of daylight, these readings are a fair sample, chosen from about three times as many Some rat holes opened outside buildings, others inside stables and granaries Individual observations differ much from one another, but in every case the saturation deficiency was less in the hole than outside it It was never possible to pass a rubber tube more than 3 feet down a hole had one reached further the saturation deficiency would doubtless have been still less

It is well known that commerce, especially in cereals, is responsible for the spread of rats and their fleas It is clearly of importance to obtain information about the climate in a heap of straw, or a sack of grain, or among a number of bales of goods I studied these problems in two places Fig 4 shows facts collected in Jerusalem In the barn (Table I) in which ventilation was free, there was a heap of threshed oats By burying a capillary in the heap, I was able to take samples of the air among the grain, from a spot about 2 feet below the surface The samples

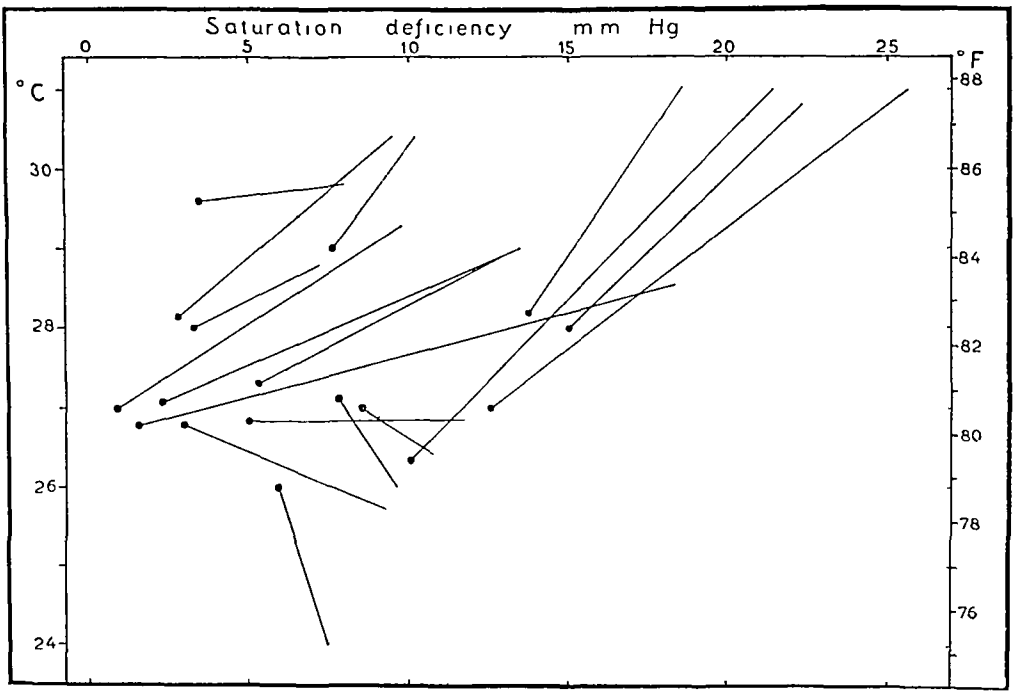

Fig 3 —Climate inside and outside rat holes, in Haifa and Jerusalem, during the hours of daylight, June and July Each line shows a different observation, the black spot indicating the temperature and saturation deficiency 2 to 3 feet down the hole, the other end of the line indicating the conditions outside

gave me the dewpoint Conditions in the barn and outside were studied by whirling psychrometers Each line in Fig 4 starts at a point which corresponds to the temperature and saturation deficiency of the outside air , it runs to a white circle, giving the conditions in the air of the barn, and ends at a black circle, corresponding to the conditions inside the heap of oats If the means of the 7 observations are taken, one finds that the temperature in the barn was 29 3°C , that in the heap 31 3°C the heap was at a higher temperature on each occasion I attribute this to the

metabolism of the grain, but one would prefer to have data recorded throughout day and night The mean vapour pressure in the grain was 16 5 mm , that in the barn 15 0 mm this also is evidence that the grain was respiring this is surprising, for it was not freshly harvested, and its water content was only 7 9 per cent when

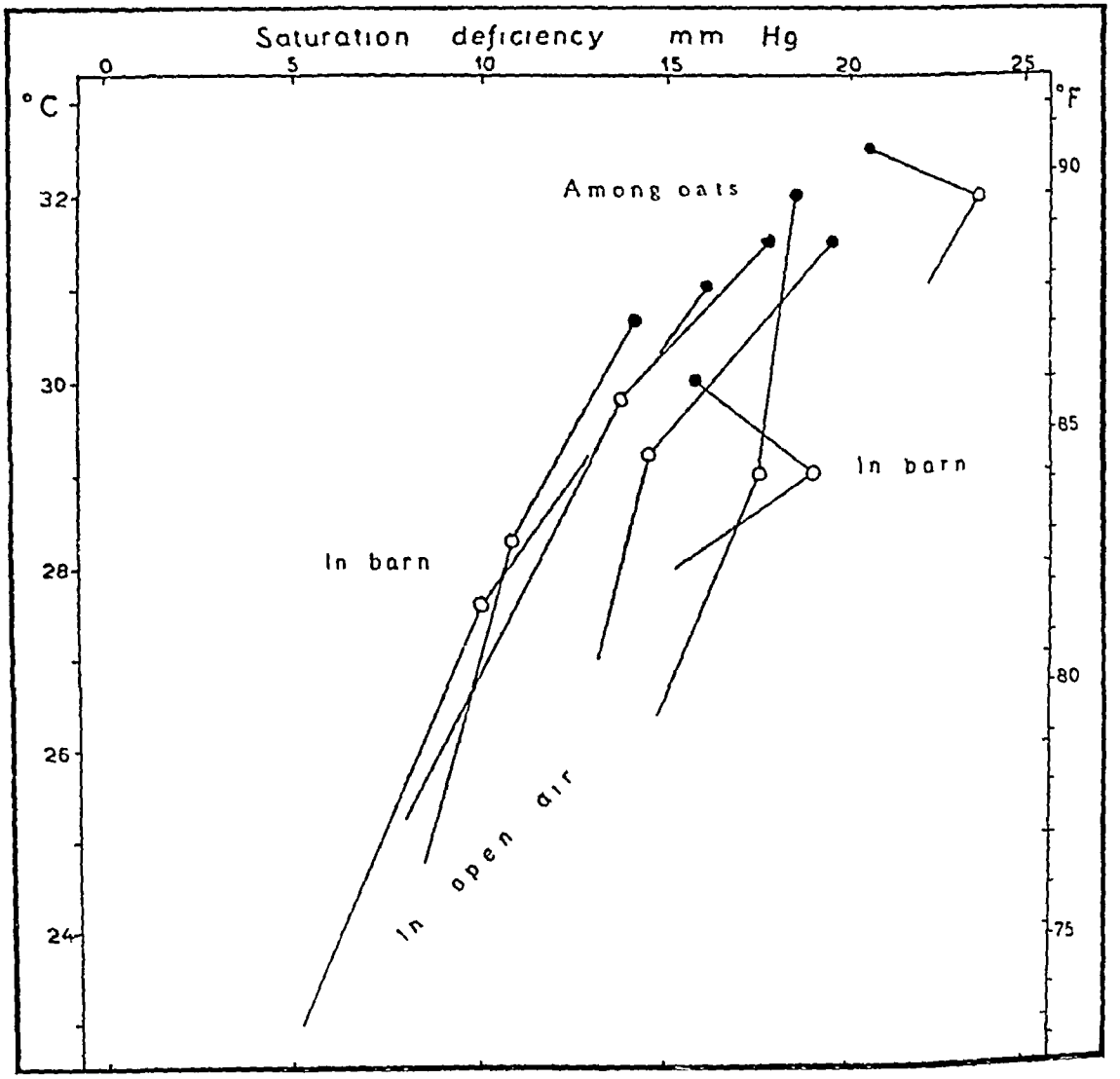

Fig 4 —Simultaneous observations on climate in the open air, in the barn, and inside a heap of grain on the floor of the barn Lines connect the three observations made at the same time Jerusalem, July

dried at 100°C The temperature and the absolute humidity were greater in the grain than outside , the saturation deficiency was also greater (mean 17 6 mm in grain, 15 7 mm in barn)

Observations on a similar plan were made at Haifa, in a large galvanized iron warehouse* (free ventilation under roof), and in the small cavities which existed between sacks of lentils the sacks were stacked in a regular manner, and I was able to insinuate a rubber tube down to about 5 feet from the top of the stack Taking 5 observations on different days, I found that the absolute humidity was consistently less among the sacks than in the general atmosphere of the warehouse presumably, therefore, the lentils were absorbing water from the atmosphere The saturation deficiency was also less among the sacks, at the time the observations were taken I was not able to record temperature day and night, either in the warehouse or among the sacks I cannot attach much importance to the values

IV Discussion

The present paper is not principally a record of fact, but a discussion of a point of view It is agnostic rather than informative

Temperature—There is a considerable difference between the climate in a standard Stevenson's screen and that studied in other places There are many causes of this, the most important being that the screen is so constructed as to shield the instruments from the effects of radiation But every building absorbs radiant heat by day and loses it at night, and this is true also of the surface of the soil For this and other reasons, the conditions in the screen are of little interest to the biologist There is a good example of this in Fig 2 The office in Haifa was at a higher temperature than the screen This is particularly noticeable during the hours of daylight on an average over the 24 hours the office was 3°C hotter than the screen

But apart from differences between the climate in the screen and that in various buildings, there are great differences between different buildings In cellars and among masses of goods in a warehouse, we may assume that the range of temperature is very little But in situations immediately under a roof or in a small space in the thickness of a south wall, it is certain that the daily maximum will be very high and it is possible that the range would be found to be greater than that in a screen

Humidity—In some of the places in which records were taken, the differences in humidity are simple If one contrasts the figures for the screen and the office (Fig 2), it will be seen that the absolute humidity is the same in both and that it is nearly the same at all times of day and night the vapour pressure is always close to 18 mm of mercury This is doubtless due to the free ventilation of the office it received an average sample of the air which was circulating over Haifa, there was nothing to add water to it In this simple case, as the temperature is different in the

* I am indebted to the manager of the Levant Bonded Warehouses for permission to make observations in this place

two places at any particular time though the absolute humidity is the same, the relative humidity and the saturation deficiency will be different But the climate in many of the places studied is complicated by the fact that water is evaporating, or is being absorbed from the air The facts collected in the stable at Haifa (Fig 2) provide an example of this Owing to the evaporation from the ground and from the animals, the vapour pressure in the stable was about 21 mm of mercury or higher Evaporation was also taking place in the rat holes (Fig 3), so that their absolute humidity was higher than that of the outside air This is remarkable, because the readings were taken at the height of the dry season when no appreciable rain had fallen for many weeks moreover, I was only able to take measurements of the climate 2 or 3 feet down a rat hole Had my tubes penetrated further, I should presumably have found a still higher absolute humidity

The data recorded from inside the heap of oats (Jerusalem) and from between the sacks of lentils (Haifa) appear to be contradictory The vapour pressure among the oats was consistently higher than it was in the air outside them, whereas among the sacks of lentils the vapour pressure was less than in the surrounding air The data show that the differences are consistent It appears probable that the oats were giving off water as a part of their vital activities, and that the lentils, which had been dried in the sun in the hills and were awaiting shipment in the damper climate of the coast, were absorbing moisture from the air with which they had not yet reached equilibrium But whatever the explanation, it is important to notice the differences which were observed and to realize how complicated are the water relations of organic materials whether living or dead If the metabolism of a heap of dried oats is sufficient to raise the absolute humidity and also apparently the temperature inside the heap, this affects the relation between trade in cereals and the distribution of particular sorts of rat-flea

Biology—We have not yet sufficient facts about the effect of temperature and humidity on rat-fleas, at various stages in their life history The combinations of conditions which are unfavourable, favourable and optimal require precise delimitation we also need to know the duration of various stages at a number of different temperatures But the facts here set out show that standard meteorological data give a false impression of the climate It is probable that nearly all the environments are hotter than the screen, and that the difference in temperature is enough to increase the rate of multiplication of fleas The fact that evaporation takes place in cellars and rat holes, even in the middle of the dry summer, is very important Taken in conjunction with the fact that high diurnal temperatures do not occur, this means that the saturation deficiency is never very great the climate is therefore favourable to rat-fleas, at all stages, though the screen climate at midday is so hot and dry that it would be rapidly fatal, at least to the larvæ

Control of climate—It appears that investigations of this sort might lead to important results in practice In one and the same town the existence of fleas

might be impossible in one position though they might be multiplying rapidly in another It should not be difficult to construct warehouses so as to secure a particular climate inside them If, for instance, the roof is flat and black, and if cereals are stored close to it, they will be heated and dried by the sun's heat The same result might be obtained with a glass roof, especially if a glass was chosen which was particularly permeable to infra-red If, moreover, the warehouse is solidly constructed and ventilated by louvres which can be opened or shut at will, then water evaporated from the goods or cereals can be liberated into the air whenever the absolute humidity is greater in the warehouse than outside In this way the water-content of the merchandise, and of the air spaces among it, will be reduced If the temperature is also raised, the saturation deficiency in every part of the granary will become very great This will prevent the breeding of fleas and incidentally do much to reduce the infestation of the stored products by beetles, moths, etc I do not suggest that this method will rid a place of plague, but it seems clear that the plague-free part of the year, which is characteristic of the Mediterranean and of Northern India, could be greatly prolonged in warehouses constructed in the manner here suggested One cannot hope that ordinary village granaries will be built in this way, but the plan should be applicable to grain elevators and warehouses at ports and at railway junctions The suggestion will become practicable when we know more of the climate inside bags of corn or bales of cotton , we also require more precise data about the effects of physical factors on all stages of rat-fleas

V Summary

The paper contains a number of records of temperature and humidity collected in Palestine at the hot dry time of year, from various places in which rats and fleas lived

Methods appropriate for this type of investigation are described, and the different scales on which humidity may be measured are discussed Thermohygrographs were exposed in stables, barns, etc , in addition observations were made from time to time, but not with recording instruments, on the temperature and humidity in rat holes and among heaps of cereals

The temperature in the small environments is frequently higher than that in a meteorological screen This is presumably due to the fact that any building, and also the surface of the ground, absorbs radiant heat from the sun In the rat holes, etc , the temperature is also doubtless very stable The conditions of humidity were shown to be very different in different places In a well ventilated office, the absolute humidity was the same as in the screen The saturation deficiency and the relative humidity in these circumstances are dependent on the temperature But in places in which evaporation is taking place, the absolute humidity is higher than it is outside This was shown to be so in a cellar, a stable and a number of

rat holes In such situations inasmuch as the temperature and the absolute humidity are both different from those in the meteorologist s screen, one cannot generalize about the saturation deficiency in the majority of these places it was less than in the screen These environments are therefore on the whole more favourable to flea-breeding than one would suppose from the study of the ordinary meteorological data

It is suggested that, if further facts were available and if more were known of the precise relation between climate and fleas at various stages, it would become possible to construct buildings within which the climate would be unfavourable to fleas If full use of solar radiation were made, it should be quite possible to make the climate inside such a warehouse unlike that outside, and to maintain a considerable difference In this way, cereals, unginned cotton, etc , could be rendered free of rat-fleas

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Ind Jour Med Res, XX, 1, *July*, 1932

AN UNUSUAL TYPE OF PARASITIC INFECTION OF FOREARM

BY

A VASUDEVAN, MB & BS,

Curator of Pathological Museum, Medical College, Madras

[Received for publication, March 23, 1932]

A PORTION of tissue from a lesion in the forearm of a person was received for pathological examination from Ceylon Unfortunately it has not been possible so far to obtain any history of the case

Naked eye appearance

An oblong firm mass roughly $2\frac{1}{2} \times 1$ inches Its surface is finely granular and of a dark slaty colour and has the appearance of a partly healing ulcer It is dense and fibrous and cuts with some resistance The cut surface is of a white colour and shows numerous yellowish brown foci, varying in size from a pin's head to that of a mustard seed, resembling tiny abscesses

Microscopical appearances

Examined under the low power, the section is seen to consist, in main, of a mass of fibro-fatty tissue with an ulcerated surface The ulcer is partly covered with stratified epithelium with a few sweat and sebaceous glands The rest of the section consists of a mass of fibrous subcutaneous tissue and shows marked round cell infiltration both diffuse and focal, and thickened blood vessels Here and there are seen giant cell systems, though isolated giant cells are also seen

Examined under the high power, the giant cell systems are not unlike those seen in tuberculous infection and consist of a central multinucleated giant cell with an outer zone of endotheloid cells and a peripheral zone of small round cells Close to some of these giant cells are seen the organism to be described hereafter

Numerous suppurative foci are seen scattered here and there These show in the centre a fibrinous exudate, red cells, and leucocytes mostly polymorphonuclears

Explanations of Plate VIII

Fig 1 Early stage of the organism embedded in a mass of pus cells inside an abscess cavity Zeiss —oc × 8, obj × 40

„ 2 Same as Fig 1, but more magnified, showing the nucleus, nucleolus, protolasmic strands and granules and the capsule Zeiss —oc × 8, obj × 90

„ 3 The organism inside a giant cell system
(*a*) The organism
(*b*) Giant cell
Zeiss —oc × 8, obj × 40

„ 4 The organism showing a marginal nucleus Zeiss —oc × 8, obj × 90

„ 5 Oblique section of an elongated organism with the protoplasmic granules and a double contoured hyaline capsule Zeiss —oc × 8, obj × 90

„ 6 Two organisms side by side inside an abscess Zeiss —oc × 8, obj × 90

Plate VIII

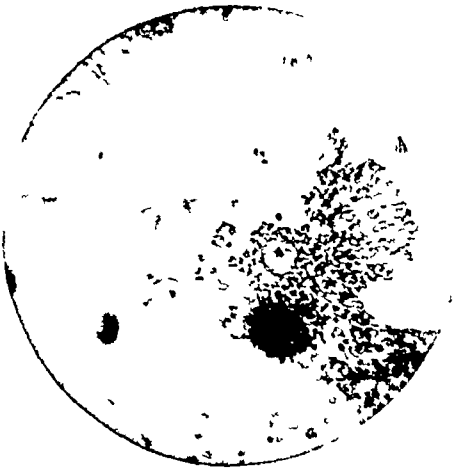


Fig 1

Fig 2

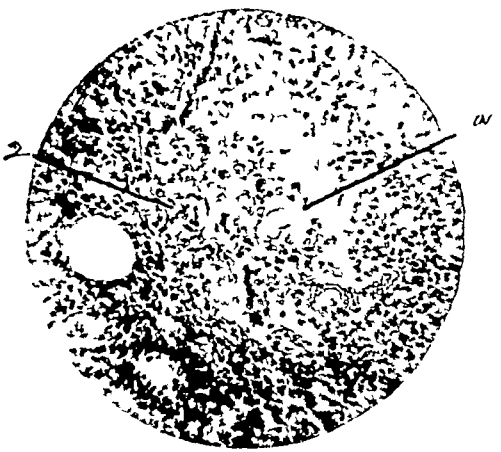


Fig 3

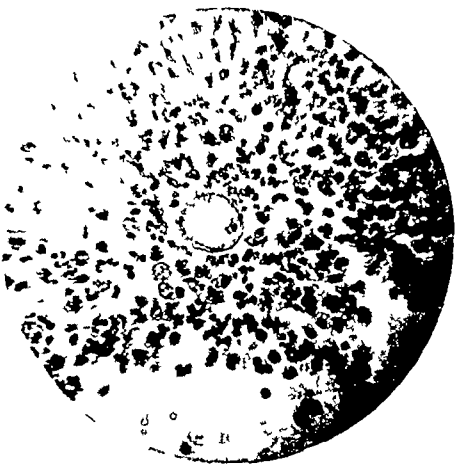


Fig 4

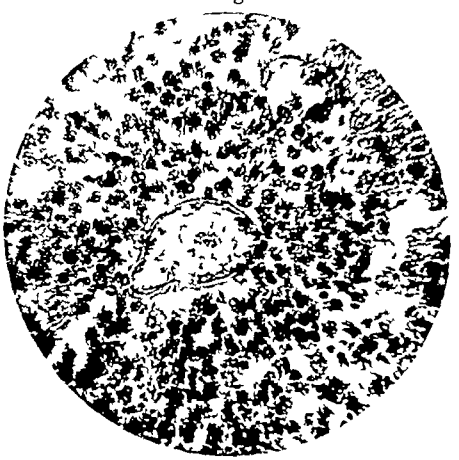


Fig 5

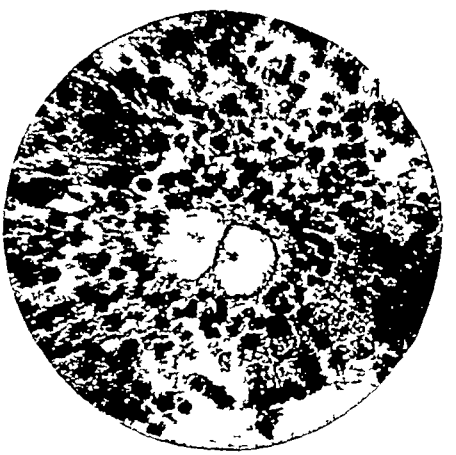


Fig 6

SEASONAL INCIDENCE OF TERTIAN, SUBTERTIAN AND QUARTAN INFECTIONS

BY

M O T IYENGAR,

Medical Entomologist, Travancore

[Received for publication, March 31, 1932]

THE writer made a study of the seasonal incidence of malarial infection by a systematic examination of blood films from malarial patients in an intensely endemic area in Bengal These observations were continued without interruption for a period of 16 months from June 1930 and over 2,600 blood films were examined during this period The material for this study was obtained from patients attending the field treatment centre of the Bengal Public Health Department at Singur in Hooghly District and the writer is much indebted to the Director of Public Health, Bengal, for his courtesy and facilities offered in connection with this work Thin films were taken from patients with a clinical history of malaria, and who did not have any quinine treatment for at least ten days previously The films were stained and examined in the laboratory at Calcutta and at least 100 microscopic fields were examined before the film was declared negative

In view of the fact that the blood films were taken from selected cases with definite symptoms of malaria and who had no quinine treatment previously, a large proportion of the blood films were positive even in thin film examination Out of a total number of 2,652 films examined in this series, 1,876, or 71 per cent, were positive for malaria parasites

An analysis of the 1,876 positive films shows that 784 were positive for *Plasmodium vivax*, 943 for *P falciparum* and 271 for *P malariæ* Taking the sum total of the observations the relative frequency of the three infections is as follows —

P vivax	*P falciparum*	*P malariæ*
41 8	50 3	14 4

1 *Seasonal incidence of the infections*

A summary of the finding in regard to the seasonal incidence of tertian, subtertian and quartan infections and of the respective gametocyte carriers is furnished in Table I It should be noted that in this table, mixed infections have been included under the component heads, for example, a mixed tertian and subtertian infection is included under *P vivax* as also under *P falciparum* As such, the totals of the first columns under each species may amount to more than the number of positives for the month The incidence of mixed infections is discussed separately at a later part of the paper

TABLE I

Period	Total number of positive films	*Plasmodium vivax*				*Plasmodium falciparum*				*Plasmodium malariæ*			
		Positive for *P vivax*	Percentage	Positive for gametocytes	Percentage	Positive for *P falciparum*	Percentage	Positive for gametocytes	Percentage	Positive for *P malariæ*	Percentage	Positive for gametocytes	Percentage
January	153	55	35 9	36	23 5	73	47 7	31	20 3	30	19 6	13	8 5
February	106	62	58 5	30	28 3	30	28 3	8	7 5	18	17 0	12	11 3
March	106	71	67 0	34	32 1	27	25 5	12	11 3	15	14 2	8	7 5
April	111	71	64 0	33	29 7	36	32 4	24	21 6	11	10 0	8	7 2
May	104	38	36 5	17	16 3	52	50 0	23	22 1	21	20 2	16	15 4
June	181	72	40 0	36	20 0	62	34 4	30	16 7	60	33 3	42	23 3
July	183	89	48 6	22	12 0	68	37 2	26	14 2	42	22 9	30	16 4
August	320	106	33 1	47	14 7	211	66 0	82	25 6	35	10 9	23	7 2
September	206	61	29 6	37	18 0	145	70 4	67	32 5	13	6 3	4	1 9
October	132	38	28 8	23	17 4	95	72 0	39	29 6	14	10 6	11	8 3
November	133	58	43 6	29	21 8	69	51 9	27	20 3	6	4 5	4	3 0
December	141	63	44 7	37	26 2	75	53 2	21	14 9	6	4 2	3	2 1
TOTAL	1,876	784	41 8	381	20 3	943	50 3	390	20 8	271	14 4	174	9 3

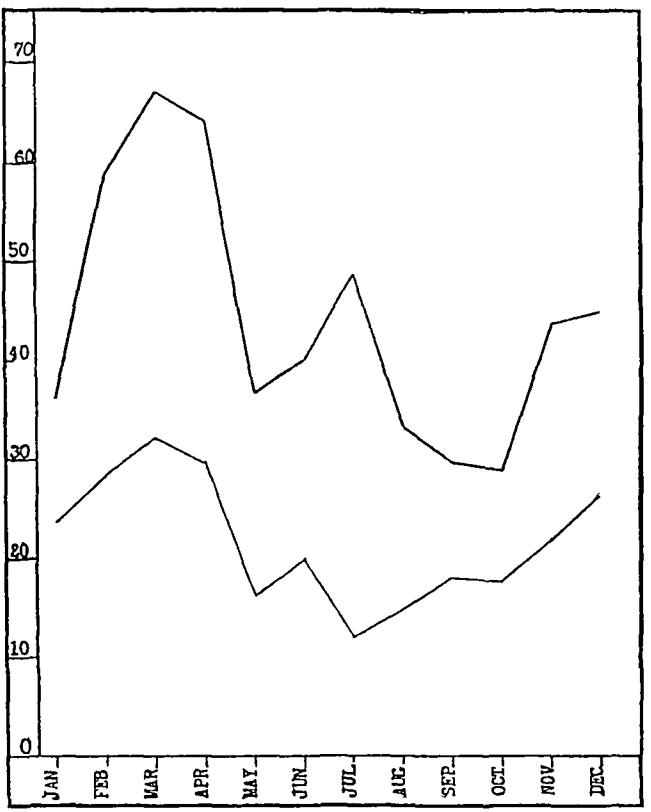
Seasonal distribution of Plasmodium vivax *infection*

The monthly incidence of infections with *P vivax* furnished in Table I is represented graphically in Chart 1 *P vivax* incidence ranges between 29 per cent and 67 per cent From a low level in January, the curve rises rapidly in

February and reaches its highest level for the year during March and April During this period, relapses are very common and these spring relapses consist very largely of *P vivax* infections With the onset of summer, the curve of *P vivax* incidence falls sharply and reaches a 37 per cent level in the month of May The curve has a

CHART 1

Seasonal incidence of P vivax *infections*



The thick line represents the percentage of *P vivax* infections and the thin line, the percentage of *P vivax* gametocyte carriers to the number of total positives during the month

small rise in July after which it reaches a low level in September and October Towards the close of the year, the incidence of *P vivax* infections rises during November and December

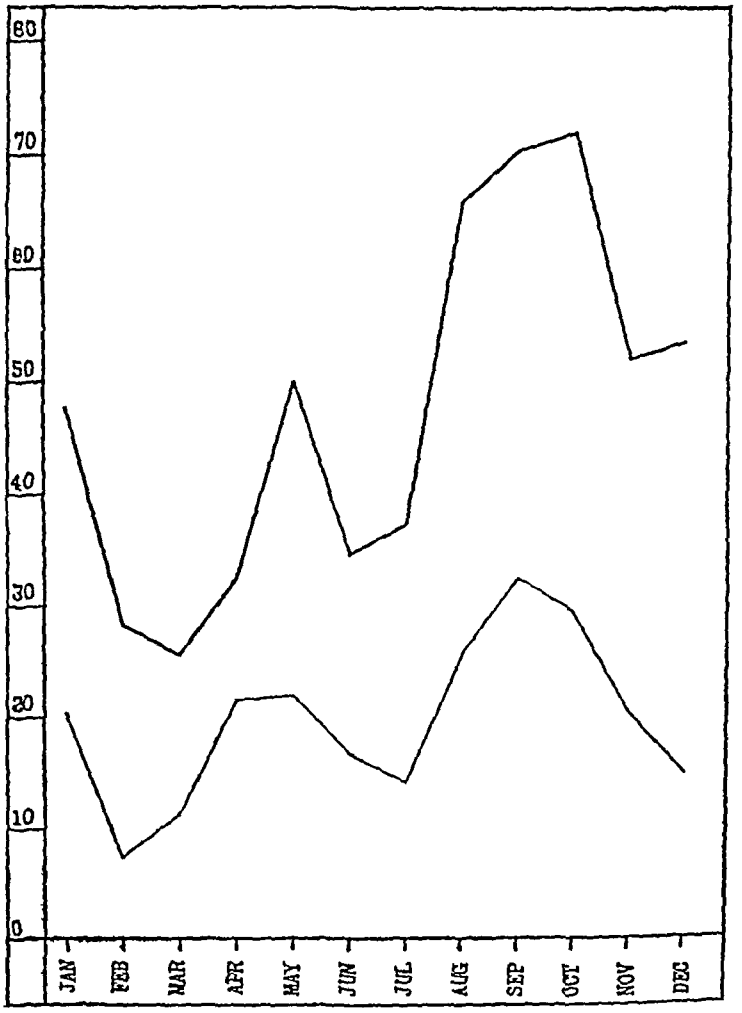
The incidence of *P vivax* infections with gametocytes is also represented graphically in Chart 1 This curve follows the general trend of the main curve of *P vivax* incidence

Seasonal distribution of Plasmodium falciparum *infection*

The monthly incidence of *P falciparum* infection is represented graphically in Chart 2 It ranges between a maximum of 72 per cent and a minimum of 25

CHART 2

Seasonal incidence of P falciparum *infections*



The thick line represents the percentage of *P falciparum* infections, the thin line represents the percentage of gametocyte carriers to the number of total positives for the month

per cent The incidence of this infection is low during February, March and April In May, there is a small rise but this is followed by a decline in the months June and July In August, the curve rises suddenly to a 66 per cent level and during

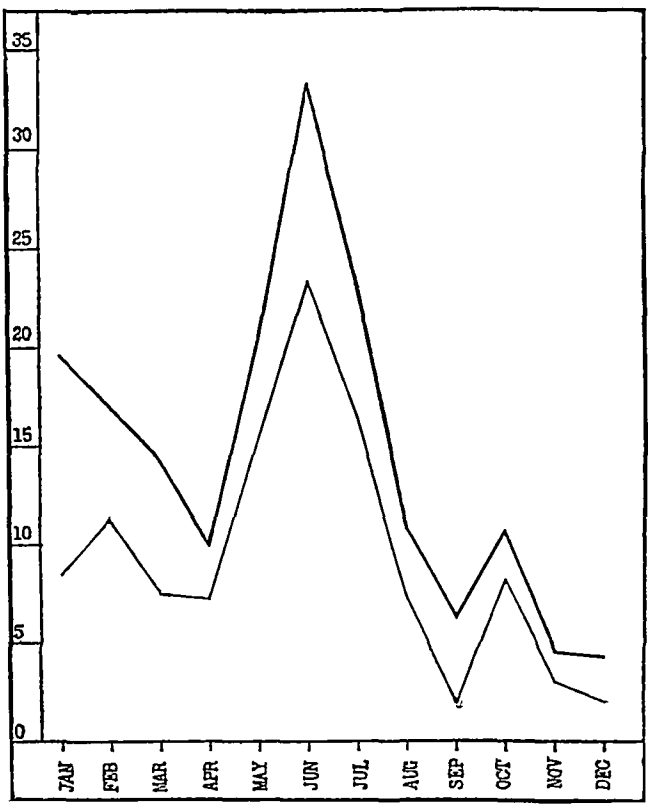
September and October it is above 70 per cent After October the curve declines rapidly and reaches its lowest incidence during the winter months The curve of incidence of *P falciparum* gametocyte infection follows the curve of *P falciparum* incidence

Seasonal distribution of Plasmodium malariæ *infection*

Compared with the incidence of the two other infections, *P malariæ* infections are less common, but they are by no means rare At certain seasons of the year,

CHART 3

Seasonal incidence of P malariæ *infections*



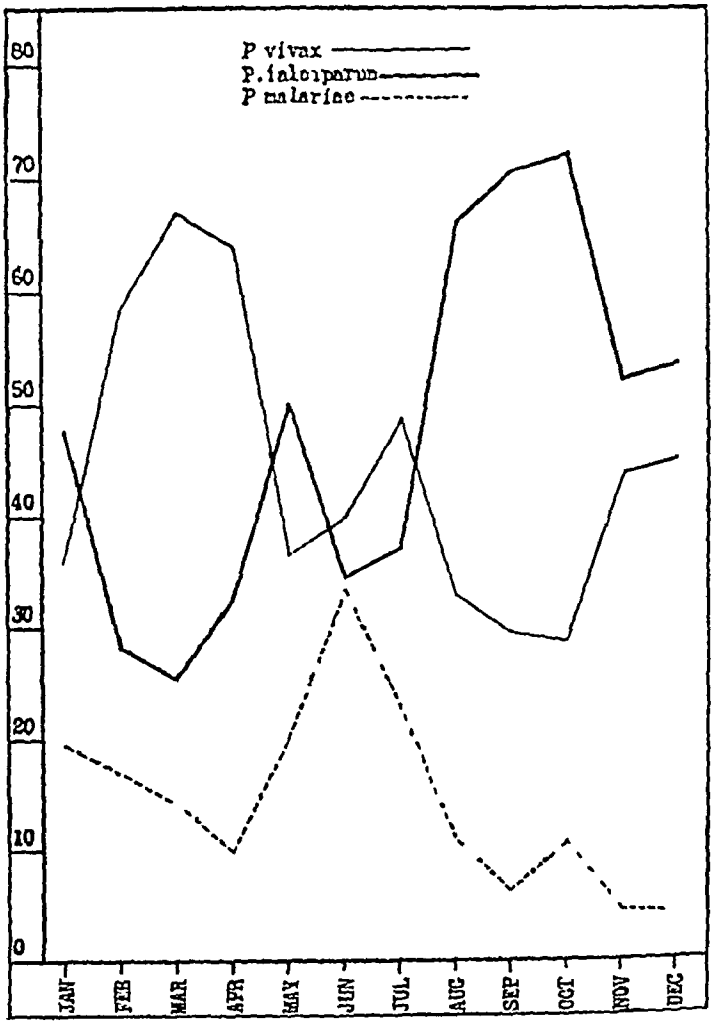
quartan infections are of fairly common occurrence The range of incidence is smaller than those of the two other infections, being between a minimum of 4 per cent and a maximum of 33 per cent The seasonal distribution of quartan infection is represented graphically in Chart 3 The curve starts at a 20 per cent level in

January and declines gradually to a 10 per cent level in April After April, the curve rises rapidly and reaches a 33 per cent level in June

The season of maximum prevalence of *P malaria* infection is during May to July After August till the close of the year, the incidence of the infection is low, except for a small rise to 11 per cent in October The curve of *P malariæ* gameto cyte infection follows very closely the curve of *P malariæ* incidence

CHART 1

Comparative incidence of the three infections



For purposes of comparison the seasonal distribution of the three infections is represented graphically on the same scale in Chart 4 Subtertian infections are high during August, September and October, a period when tertian infections are low Tertian infections are most common during February, March and April when

TABLE II

Period	Number of positive films	Single infections			Mixed infections				Total number of mixed infections	Percentage of mixed infections
		P vivax	*P falciparum*	*P malariæ*	*P vivax* and *P falciparum*	*P vivax* and *P malariæ*	*P falciparum* and *P malariæ*	*P vivax*, *P falciparum* and *P malariæ*		
January	153	50	70	28	3	2	0	0	5	3 3
February	106	58	29	15	1	3	0	0	4	3 8
March	106	64	24	11	3	4	0	0	7	6 6
April	111	64	30	10	6	1	0	0	7	6 3
May	104	31	47	19	5	2	0	0	7	6 7
June	181	60	56	51	4	8	1	0	13	7 2
July	183	75	58	34	8	6	2	0	16	8 7
August	320	80	181	34	24	1	5	1	31	9 7
September	206	48	134	11	11	2	0	0	13	6 3
October	132	23	83	11	12	3	0	0	15	11 4
November	133	58	69	6	0	0	0	0	0	0 0
December	141	60	72	6	3	0	0	0	3	2 1
TOTAL	1,876	671	853	236	80	32	8	1	121	6 4

subtertian infections are low Quartan incidence is small in comparison with the incidence of tertian and subtertian infections Quartan infections are highest during May, June and July The observations on the incidence of the three infections in this area (Hooghly District, Bengal) show distinct seasons of prevalence of the three infections, the quartan season in June, the subtertian season in September and the tertian season in March

2 *Incidence of mixed infections.*

In the cases studied, mixed infections were by no means rare A statement is furnished above (Table II) giving the incidence of single infections and of mixed infections, the character of the mixed infections and their seasonal distribution

Taking the sum total of these observations 121 mixed infections were observed in thin film examination in a total of 1,876 positive films, working to a gross mixed infection rate of 6 4 per cent During the period November to February, mixed infections are infrequent They are most common during June to October

The relative incidence of the different combinations of mixed infections, taking the total number of mixed infections as 100, is furnished below —

P vivax and *P falciparum*	*P vivax* and *P malariæ*	*P malariæ* and *P falciparum*	*P vivax*, *P malariæ* and *P falciparum*
66 1	26 4	6 6	0 8

The most common type of mixed infection observed is *P vivax* with *P falciparum*, which comprises 66 1 per cent of the total mixed infections observed Next to this come mixed infections with *P vivax* and *P malariæ*, which comprise 26 4 per cent Mixed infections with *P malariæ* and *P falciparum* are less frequent and mixed infections showing the presence of all the three species are very rare

3 *Incidence of gametocyte carriers in the three infections*

The following statement is a summary of the incidence of gametocyte carriers in the three infections —

	P vivax	*P falciparum*	*P malariæ*
Number of positive films	784	943	271
Number of films with gametocytes	381	390	174
Percentage of films with gametocytes	48 6	41 4	64 2

The highest incidence of gametocyte carriers occurs in *P malariæ* infections, 64 2 per cent of the films positive for *P malariæ* were observed to have gametocytes In *P vivax* infections, 48 6 per cent of the positives had gametocytes The lowest incidence of gametocyte carriers was observed in *P falciparum* infections in which 41 4 per cent were positive for gametocytes

These results compare well with the figures obtained by Kligler and Reitler (1928) while working in Palestine Their results are reproduced below —

Kligler and Reitler's (1928) *findings*

P vivax			*P falciparum*			*P malariæ*		
Cases positive	Gameto cyte carriers	Percent age	Cases positive	Gameto cyte carriers	Percent age	Cases positive	Gameto cyte carriers	Percent age
88	38	43 2	127	46	36 2	59	39	66 1

These authors' findings are that quartan infections have the highest incidence of gametocytes and that subtertian infections the least The gametocyte carrier rates of Kligler and Reitler quoted above, namely 66 1, 43 2 and 36 2 for quartan, tertian and subtertian infections respectively correspond fairly closely with the present author's figures of 64 2, 48 6 and 41 4 respectively Knowles and Senior-White (1930, p 306) conclude from a summary of the results of workers all over the world, that the relative incidence of films with gametocytes is lowest in *P falciparum* infections and highest in *P malariæ* The present findings confirm their conclusions

4 *Seasonal incidence of gametocyte carriers*

The incidence of gametocyte carriers to the total positive cases is given in Table I and represented graphically in Charts 1, 2 and 3 These curves tend to follow the incidence curve of the respective species But they do not bring out the individual variations in the incidence of gametocyte carriers to the total positives of the same species This is dealt separately in this section

P. vivax —

Table III furnishes details of the seasonal distribution of gametocyte incidence of *P vivax* —

TABLE III

Plasmodium vivax

Period	Number of positive films	Number of films with gametocytes	Percentage of films with gametocytes
January	55	36	65 5
February	62	30	48 4
March	71	34	47 9
April	71	33	46 5
May	38	17	44 7
June	72	36	50 0
July	89	22	24 7
August	106	47	44 3
September	61	37	60 7
October	38	23	60 5
November	58	29	50 0
December	63	37	58 7
TOTAL	784	381	48 6

The incidence of tertian gametocytes is generally high during the period September to January and it is lower during the rest of the year It is at its lowest during July

P falciparum —

The seasonal distribution of gametocyte incidence is represented graphically in Chart 5 The details of the results of the observations are furnished in Table IV —

TABLE IV

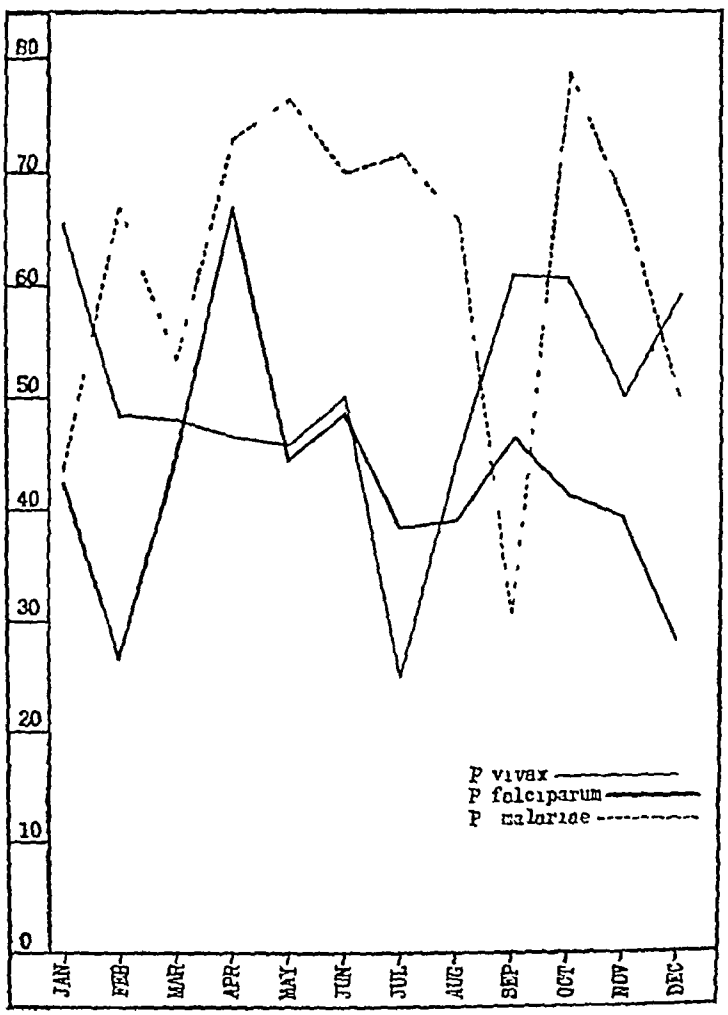
Plasmodium falciparum

Period	Number of positive films	Number of films with gametocytes	Percentage of films with gametocytes
January	73	31	42 5
February	30	8	26 7
March	27	12	44 4
April	36	24	66 7
May	52	23	44 2
June	62	30	48 4
July	68	26	38 2
August	211	82	38 9
September	145	67	46 2
October	95	39	41 1
November	69	27	39 1
December	75	21	28 0
TOTAL	943	390	41 4

The incidence of *P falciparum* gametocytes is high during two seasons, March to June and September to November.

CHART 5

Incidence of Gametocyte carriers in the three infections



P malariæ —

The seasonal distribution of gametocyte carriers is shown graphically in Chart 5 Details of the findings are furnished in a statement below (Table V) .—

TABLE V

Plasmodium malariæ

Period	Number of positive films	Number of films with gametocytes	Percentage of films with gametocytes
January	30	13	43 3
February	18	12	66 7
March	15	8	53 3
April	11	8	72 7
May	21	16	76 2
June	60	42	70 0
July	42	30	71 4
August	35	23	65 7
September	13	4	30 4
October	14	11	78 6
November	6	4	66 7
December	6	3	50 0
TOTAL	271	174	64 2

A high incidence of *P malariæ* gametocyte carriers occurs during April to July and in October

5 *Intensity of infection*

Observations were made on the intensity of the infection, by counting the number of parasites observed in 100 microscopic fields of the thin film, out of a random selection of positive slides A fairly large number of slides were examined

in this manner and the results have been tabulated to furnish the average number of parasites per film, the average number of gametocytes per film, and the gametocyte ratio (proportion of gametocytes to total parasites observed) These counts are subject to some variation as a result of difference in the thickness of the films and the number of corpuscles examined but in view of the fact that the observations cover a large number of films and the films were all taken by the same person, the error as a result of such variations may not be considerable The results of these observations are discussed below —

Taking all the records together, the average incidence of malaria parasites of the three species in the blood films examined is given in a comparative statement below —

Average number of parasites per positive film (100 *fields*)

P vivax	*P falciparum*	*P malariæ*
33 1	27 9	11 4

These figures, based on a large number of observations, show that on the average *P malariæ* infections have the least number of parasites, and that *P vivax* has the heaviest infection

These results are not in conformity with the general impression of workers, namely, that '*P falciparum* infections tend to show a much heavier incidence of parasites in the film than do infections with *P vivax* or *P malariæ*' (Knowles and Senior-White, 1930, p 300) The heavier average infestation (intensity of infection) observed in tertian infections in this series, as compared with the average infestation in subtertian infections is not in a line with the common belief The average figure of 27 9 parasites per 100 fields in *P falciparum* infections may appear to be low for subtertian infections Even in the present series of observations, very heavy infections with subtertian parasites have been observed (Table VI) But while working over a large series extended over a whole year and striking the average, tertian infections show a higher average infestation than *P falciparum* infections There are other likely factors contributory to the comparatively lower average infestation observed in *P falciparum* infections and a higher infestation in *P vivax* infections It should be remembered that these figures represent ambulatory cases only, as the blood films were taken only from patients attending the treatment centre personally As such, the more intensive infections with *P falciparum* in view of the too severe reactions that they produce, render the patients unable to attend the treatment centre personally and, therefore, the more intensive infections may not be represented in sufficiently large numbers in these records This factor tends to lower the average infestation in *P falciparum* infections There is yet

another factor that helps to give a high infestation figure for *P vivax* infections In the hyperendemic area where these investigations were carried out, low infections with *P vivax* are not represented sufficiently in the field treatment centre, as mild tertian infections do not produce sufficiently severe symptoms to induce the patient to go to the centre for treatment This would tend to raise the infestation figure in *P vivax* infections These factors should be considered together in judging the degrees of infestation in the three infections In regard to *P malariæ* infections the author's findings confirm Knowles and Senior-White's view 'In general, perhaps the scantiest infections are those with *P malariæ*'

The following table gives the frequency of the varying degrees in the intensity of infection observed in the three species based on the number of parasites seen in 100 microscopic fields of thin films —

TABLE VI

Classification of blood films according to the degree of infestation

	1–10 parasites	11–25 parasites	26–50 parasites	51–100 parasites	101–200 parasites	201–300 parasites	301–400 parasites	401–500 parasites	501–600 parasites	601–700 parasites	TOTAL
P vivax	284	193	120	87	31	10	5	2			732
P falciparum	384	213	105	59	23	6	5	1	1	1	798
P malariæ	144	87	15	1							247

Infections with *P malariæ* almost invariably have a low infestation, over 92 per cent of the films examined had 1 to 25 parasites in 100 fields The heaviest infections were observed in *P falciparum* infections

Considered from the point of view of the average number of gametocytes per film, *P vivax* has the highest number of gametocytes per film while *P malariæ* has the least This follows the same order as was observed in the average number of parasites

Average number of gametocytes per positive film

P vivax	*P falciparum*	*P malariæ*
45	38	29

The average number of gametocytes is least in *P malariæ* but when compared to the total number of parasites, the incidence of gametocytes to the total number of parasites is much higher in *P malariæ* than in *P falciparum* or in *P vivax* The statement below furnishes the respective percentages of the number of gametocytes to the total parasites observed in the three infections

Gametocytes ratio

P vivax, per cent	*P falciparum*, per cent	*P malariæ*, per cent
13 5	13 5	25 5

Ratio of asexual to sexual forms

The ratios of asexual forms to sexual forms in the three infections according to the observations of Kligler and Reitler (1928) are as follows —

P vivax 2 5 1

P falciparum 3 1 1

P malariæ 1 6 1

In the present series of observations, the ratios obtained are

P vivax 6 4 1

P falciparum 6 4 1

P malariæ 2 9 1

These figures show a very much lower incidence of sexual forms to asexual forms in each of the three species than those observed by Kligler and Reitler, but, in common with their observations, the results show that *P malariæ* has the highest incidence of sexual forms

Seasonal variations in the intensity of infection

Seasonal variations in the average intensity of infection based on the number of parasites observed in 100 microscopic fields are discussed below —

P vivax —

Table VII furnishes in detail the results of observations in regard to the monthly average intensity of infection, the incidence of asexual forms and of gametocytes and the gametocyte ratio in *P vivax* infections,

TABLE VII

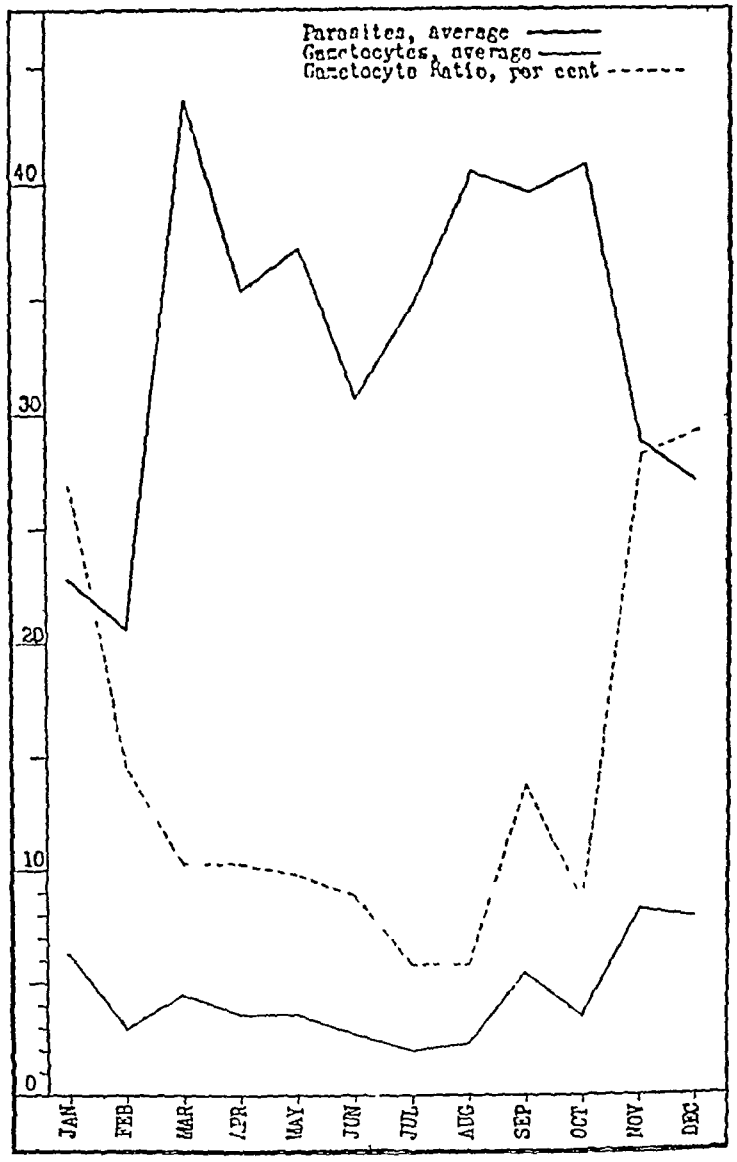
Plasmodium vivax

Period	Number of positive films examined	Total number of parasites observed	Average number of parasites per film	Total number of asexual forms observed	Average number of asexual forms per film	Total number of gametocytes observed	Average number of gametocytes per film	Percentage of gametocytes to total
January	63	1,436	22 8	1,048	16 6	388	6 2	27 0
February	72	1,485	20 6	1,268	17 6	217	3 0	14 6
March	77	3,356	43 6	3,012	39 1	344	4 5	10 3
April	74	2,609	35 3	2,341	31 6	268	3 6	10 3
May	44	1,634	37 1	1,474	33 5	160	3 6	9 8
June	52	1,589	30 6	1,447	27 8	142	2 7	8 9
July	59	2,051	34 8	1,933	32 8	118	2 0	5 8
August	77	3,121	40 5	2,941	38 2	180	2 3	5 8
September	56	2,215	39 6	1,911	34 1	304	5 4	13 7
October	25	1,020	40 8	932	37 3	88	3 5	8 6
November	63	1,811	28 7	1,302	20 7	509	8 1	28 1
December	70	1,880	26 9	1,332	19 0	548	7 8	29 1
TOTAL	732	24,207	33 1	20,941	28 6	3,266	4 5	13 5

These results are represented graphically in Chart 6 The average number of parasites per film varied between 20 and 44 The infestation is high during March corresponding to the spring rise due to tertian relapses During August to October there is another rise in the intensity of infection After October the intensity of

CHART 6

Plasmodium vivax



infection declines and is lowest during the winter months December to February The incidence of asexual forms follows closely the intensity of infection The variations in the incidence of *P vivax* gametocytes are represented on the same

chart The incidence of gametocytes does not show any marked relation to the intensity of infection and the rise or fall in the incidence of gametocytes does not correspond with the intensity of infection On the other hand, there is in *P vivax* infections a small degree of negative correlation between the two factors, namely, intensity of infection and incidence of gametocytes which is expressed by the formula —

$$r = -3452 \pm 1704$$

This does not show an appreciable degree of negative correlation but shows that there is absolutely no positive correlation between the two

But when one considers the relation between the incidence of asexual forms and the incidence of gametocytes, there is a larger degree of negative correlation between the two factors This is expressed by the formula —

$$r = -5514 \pm 1344$$

This negative correlation between the incidence of asexual forms and the incidence of gametocytes indicates that conditions which are favourable for the development of the asexual phases of *P vivax* in the human host are not favourable for the formation of gametocytes and that the production of gametocytes is more evident at a time when conditions are not quite suitable for the increase of the asexual phases of the parasite

P falciparum —

The seasonal infestation figures for *P falciparum* infections are furnished in Table VIII below

The results are represented graphically in Chart 7 The average number of parasites observed in 100 fields in *P falciparum* infections range between 8 and 49 There is a big rise in the intensity of infection during July to September, and a smaller rise in March During December to February the intensity is low The big rise during July to September corresponds to the season of maximum prevalence August to October of subtertian infections observed previously (Chart 2) The intensity of gametocytes infection has two rises corresponding to the two peaks of high infestation in *P falciparum* infections , there is a rise during April and another during September To a certain extent, the increase of gametocyte infestation varies directly as the increase of general infestation There is here a fair degree of positive correlation between the average number of parasites per film and the average number of gametocytes per film during the different months of the year This positive correlation is not very high, but its value is expressed by the formula —

$$r = +5879 \pm 1256$$

A similar degree of positive correlation is observed between the number of asexual forms of *P falciparum* and the number of gametocytes Its value is expressed by the formula —

$$r = +5012 \pm 1446$$

Table VIII.

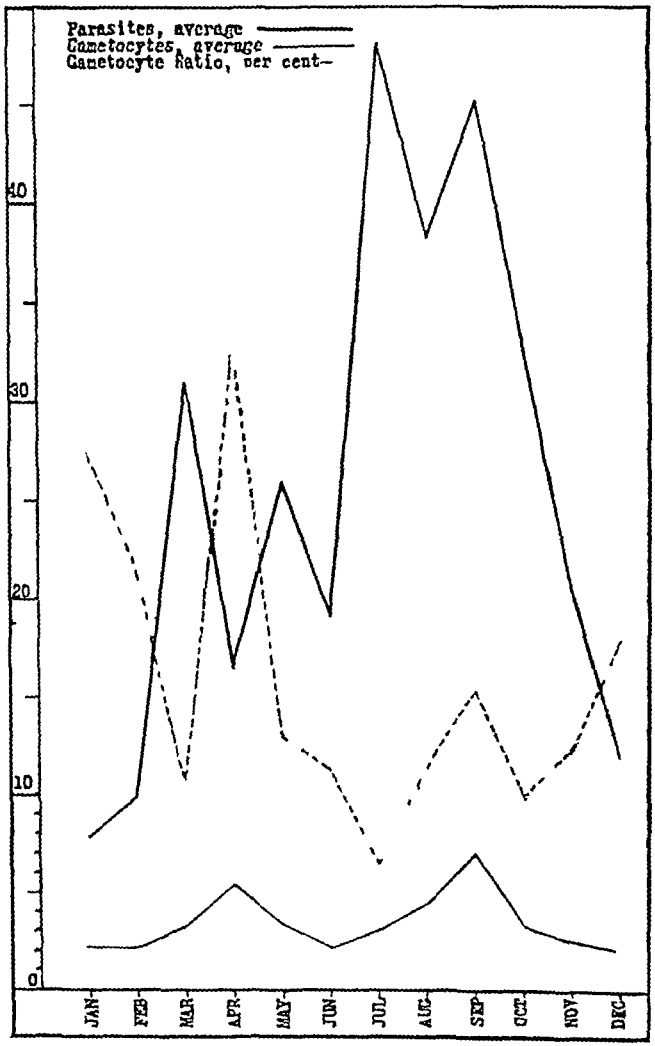
Plasmodium falciparum.

Period	Number of positive films examined	Total number of parasites observed	Average number of parasites per film	Total number of asexual forms observed	Average number of asexual forms per film	Total number of gametocytes observed	Average number of gametocytes per film	Percentage of gametocytes to total
January	80	626	7 8	456	5 7	170	2 1	27 2
February	34	335	9 9	263	7 7	72	2 1	21 5
March	30	926	30 9	829	27 6	97	3 2	10 5
April	44	732	16 6	495	11 3	237	5 4	32 4
May	58	1,501	25 8	1,307	22 5	194	3 3	12 9
June	27	516	19 1	458	17 0	58	2 1	11 2
July	38	1,823	48 0	1,709	45 0	114	3 0	6 3
August	144	5,498	38 2	4,869	33 8	629	4 4	11 4
September	127	5,729	45 1	4,851	38 2	878	6 9	15 3
October	72	2,332	32 4	2,102	29 2	230	3 2	9 9
November	66	1,338	20 3	1,174	17 8	164	2 5	12 3
December	78	915	11 7	753	9 7	162	2 1	17 7
Total	798	22,271	27 9	19,266	24 1	3,005	3 8	13 5

These results indicate that with an increase in the number of parasites in the human host, there is also some increase in the number of gametocytes, although not quite in proportion to the increase in the infestation It is seen that conditions

CHART 7

Plasmodium falciparum



favourable for the increase of asexual forms of *P falciparum* are not adverse to the formation of gametocytes

P malariæ —

Details of observations on the seasonal variations in the intensity of infection and the incidence of gametocytes are furnished in Table IX below —

TABLE IX

Plasmodium malariæ

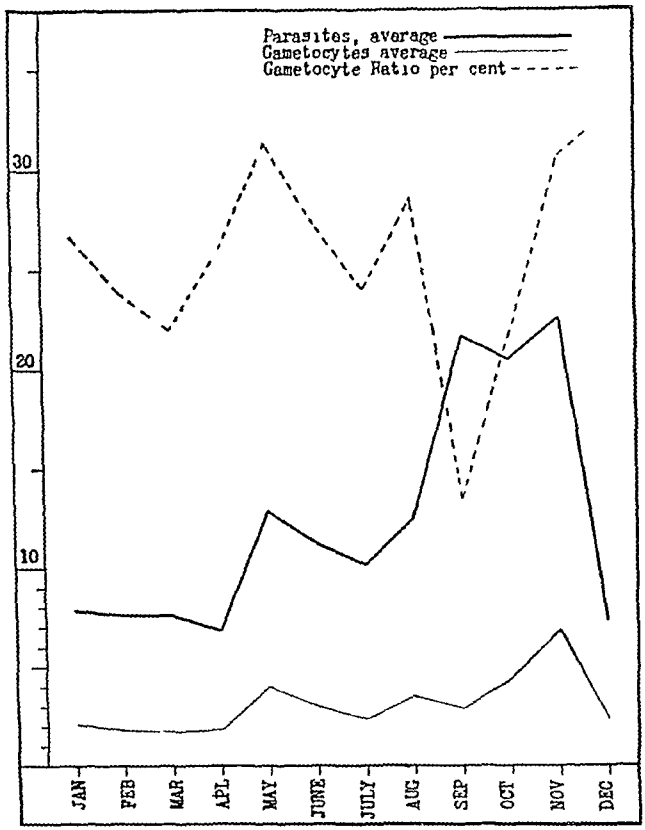
Period	Number of positive films examined	Total number of parasites observed	Average number of parasites per film	Total number of sexual forms observed	Average number of sexual forms per film	Total number of gametocytes observed	Average number of gametocytes per film	Percentage of gametocytes to total
January	31	241	7 8	177	5 7	64	2 1	26 6
February	20	151	7 6	115	5 8	36	1 8	23 8
March	22	168	7 6	131	6 0	37	1 7	22 0
April	13	89	6 8	66	5 1	23	1 8	25 8
May	26	333	12 8	228	8 8	105	4 0	31 3
June	32	357	11 2	260	8 1	97	3 0	27 2
July	31	312	10 1	237	7 6	75	2 4	24 0
August	33	410	12 4	293	8 9	117	3 5	28 5
September	11	238	21 6	206	18 7	32	2 9	13 4
October	14	285	20 4	223	15 9	62	4 4	21 8
November	8	180	22 5	125	15 6	55	6 9	30 6
December	6	43	7 2	29	4 8	14	2 3	32 6
TOTAL	247	2,807	11 4	2,090	8 5	717	2 9	25 5

The results are represented graphically on Chart 8 The average number of parasites per film ranges between 7 and 23 There is a rise in the intensity of infection during May and a bigger rise during September to November During December to April, the intensity of infection is comparatively low The

intensity of gametocyte infection varies directly as the intensity of *P malariæ* infection A high degree of positive correlation exists between the average

CHART 8

Plasmodium malariæ



number of parasites per film and the average number of gametocytes per film This positive correlation is represented by the formula —

$$r = + \ 8182 \pm 0632$$

When one considers the seasonal fluctuations in the intensity of asexual forms in *P malariæ* infections in relation to the gametocytes, there occurs a moderate degree of positive correlation between the two which is expressed by the formula —

$$r = + \ 6831 \pm 1008$$

The results show that in *P malariæ* infections a higher intensity of infection is always associated with a higher gametocyte incidence With an increase in the intensity of infection, there also occurs an increase in the incidence of gametocytes and vice versa The correlation between the two factors intensity of infection and the incidence of gametocytes is even greater in *P malariæ* infections than was observed in *P falciparum* infections

It will be observed that the three infections differ markedly in regard to the production of gametocytes In *P vivax* infections, there is a negative correlation between the average intensity of infection and the incidence of gametocytes In *P falciparum* infections there is a moderate degree of positive correlation between the two, while in *P malaria* infections the positive correlation is fairly high

Variations in the gametocyte ratio

The gametocyte ratio is not entirely a reliable factor on which conclusions may be drawn By gametocyte ratio is meant the proportion of gametocytes to the total number of parasites per cent The variations in the gametocyte ratio may not always be a true guide to the variations in the gametocyte infestation For instance, when the intensity of infection rises and the incidence of gametocytes either remains stationary, or shows a rise not entirely proportionate to the rise of the intensity of infection, the gametocyte ratio would show a fall This is illustrated by Chart 7 While there is a fair degree of positive correlation between the intensity of infection and gametocyte incidence, the gametocyte ratio varies inversely with the intensity of infection On the other hand, in Chart 6, the gametocyte ratio varies inversely as the intensity of infection and directly as the gametocyte incidence As such, the gametocyte ratio does not always represent the relation of the gametocyte incidence to the intensity of infection Much reliance cannot therefore be placed on the variations in the gametocyte ratio

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OBSERVATIONS ON DETOXICATION OF DABOIA VENOM BY HEPATIC LIPOIDS WITH A NOTE ON THE ANTI-VIPERINE POTENCY OF KASAULI ANTIVENIN

BY

MAJOR G C MAITRA, I M S,

AND

CAPTAIN S M K MALLICK, I M S

(*From the Central Research Institute, Kasauli*)

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THE destructive action of viper venom on animal tissues has always been a source of constant trouble in routine immunization of horses for raising antivenin at Kasauli It invariably leads to the formation of extensive abscesses with consequent sepsis and ill health to the animals undergoing the course of immunization Attempts have been made to overcome this difficulty on many occasions but no satisfactory treatment of daboia venom prior to its use as an antigen has been discovered Acton and Knowles (1913) reported a successful method of fixing the hæmorrhagin of daboia venom by treating it with lung tissues of rabbits They reported favourable results but their technique was not adopted at this Institute probably on account of the difficulty of making this mixture aseptic and suitable for subcutaneous injections

Maitra and Ahuja (1929) tried various treatments of daboia venom to minimize its local necrotic effect without decreasing its immunizing property They treated daboia venom with weak solution of formalin with the idea of converting it into comparatively innocuous toxoids like diphtheria toxoids They also tried combination of daboia venom with antivenin, citrated whole horse blood, weak permanganate solution and vegetable colloids such as gum acacia and tapioca The venom-antivenin mixture was the only method which produced some favourable results, i e, the horses came up to title with a dose of venom smaller than the usual

and the necrotic reaction at the site of injection was reduced to a small extent They also attempted to immunize animals by alteration of the route of administration but did not meet encouraging results (1930) Vellard (1930) reported that it was possible to reduce the virulence of viperine venoms by treating them with hepatic lipoids and that such treated venoms retained their immunizing power for a considerable time As we had felt the necessity of finding a way to do away with the local necrotic effects of daboia venom this study was taken up to see whether a preliminary treatment of the venom with hepatic lipoids could be of any practical use in the routine manufacture of combined antivenin (anti-cobra and anti-viper)

We treated a sample of daboia venom with hepatic lipoids prepared according to the following method —

A whole fresh sheep-liver cleared of fibrous and other adventitious tissues was minced aseptically as far as possible and dried at 37°C for four days After this period the dried substance was ground finely with broken glass pieces in a sterile pestle and mortar This reduced the liver substance to a fine brownish powder which was divided into two parts, one part was extracted with 100 c c of absolute alcohol and the other with 100 c c of ether for 48 hours Then the filtrates from both parts were mixed together and allowed to evaporate in the incubator at 37°C, leaving behind an amber coloured jelly-like mass This presumably contained all alcohol and ether soluble extractives which will henceforth be referred to as hepatic lipoids

Before exposing the daboia venom to the action of these lipoids it was dissolved in normal saline solution and mixed with glycerine (3 g to 1 g of venom) The mixture was evaporated to a jelly To this was added 5 g of lipoids and the combined product was incubated in a glass-stoppered weighing bottle at 37°C for two months

Vellard does not mention the time for which contact should be maintained between venom and the lipoids at 37°C but states that the desired detoxication of the venom takes place in several weeks or months After a lapse of two months we tested our treated daboia venom for sterility and it was found sterile The amount of actual daboia venom per g of mixture was calculated from the weights of its constituents and the final product

A batch of six guinea-pigs received weekly subcutaneous injections of daboia venom over a shaved area of abdomen Two of the batch received untreated daboia venom commencing from 0 1 mg Four received treated daboia venom of the same brew in the same doses Local necrosis at the site of injection was looked for in guinea-pigs 3 days after each injection The doses were gradually increased in both cases It was found that whereas the control guinea-pigs receiving untreated venom showed local necrosis and pus formation with 0 3 mg , those receiving up *to* 3 0 mg of treated venom showed no local necrosis In some of the latter cases,

however, the day after the injection local swelling was noticed, which subsided in three or four days without going to the purulent stage

Encouraged with the above observation, we began to immunize two young goats A and B in order to find out whether treated daboia venom could produce specific anti-bodies in combination of cobra venom as in practical immunization of horses at Kasauli These goats received cobra venom and treated daboia venom subcutaneously in the neck at weekly intervals, starting from cobra venom 0 1 mg and treated daboia venom 0 1 mg The weekly doses were gradually increased and the presence or absence of local reaction noted in goats 3 days after each injection It was gratifying to note the absence of local necrotic reaction throughout the immunization period which lasted for six months However, when the weekly injections were near 20 mg of each venom, we noticed local swelling but it subsided

The observation during the process of immunization of the two goats is shown in Table I

When the sera of both the goats were found of sufficient potency after a preliminary titration, they were bled and antivenin collected One c c of serum from each goat neutralized 0 5 mg of cobra venom and 0 8 mg of daboia venom when tested on pigeons After the above experiments it was decided to apply this method of immunization to one of the antivenin horses Horse No 5 was selected as it was notorious for developing unsightly abscesses, being very sensitive to the action of daboia venom The animal had been given his three months' winter rest according to the routine here The anti-viperine potency of his serum was tested and 1 c c of it failed to neutralize 1 mg of daboia venom when injected intravenously into the pigeon When immunized (*vide* Table II) by a mixture of cobra venom and treated daboia venom, 1 c c of its serum neutralized 2 mg of daboia venom and 0 5 mg of cobra venom, the latter being the only standard of potency hitherto observed at Kasauli A photograph was taken four days after the last injection (cobra venom 60 mg and treated daboia venom 60 mg) For purposes of comparison the horse on a subsequent occasion was given cobra venom 60 mg plus daboia venom 60 mg (untreated of the same brew as the treated one) in order to find out the difference in the local effects and another photograph was taken after four days of this dose A comparison of the two photographs (*see* Plate IX) will show to what extent the local reactions have been reduced by the preliminary treatment of daboia venom with hepatic lipoids

In order to find out the anti-viperine potency of our antivenin, both from goats and No 5 horse, we had to ascertain the minimum capacity of 1 c c immune serum to neutralize viper venom Moreover, the routine of testing our antivenin only against cobra venom is open to a certain amount of criticism as we cannot guarantee its exact potency against viper venom, although it is alleged to be effective against both these poisons

TABLE I

Showing the course of immunization of goats A and B by graduated doses of cobra venom (C V) and treated dabora venom (T D V)

Date	Dose	Goat A		Goat B	
		Weight	Presence or absence of local reaction at the site of injection	Weight	Presence or absence of local reaction at the site of injection
14-3-31	C V 0·1 mg + T D V 0·1 mg	40 lb	*Nil*	32 lb	*Nil*
21-3-31	C V 0·2 „ + T D V 0·2 „	40 „	*Nil*	32 „	*Nil*
30-3-31	C V 0·4 „ + T D V 0·4 „	42 „	*Nil*	34 „	*Nil*
6-4-31	C V 0·8 „ + T D V 0·8 „	45 „	*Nil*	36 „	*Nil*
13-4-31	C V 1·6 „ + T D V 1·6 „	47 „	*Nil*	39 „	*Nil*
20-4-31	C V 2 „ + T D V 2 „	48 „	*Nil*	42 „	*Nil*
27-4-31	C V 2·5 „ + T D V 2·5 „	50 „	*Nil*	45 „	*Nil*
4-5-31	C V 3 „ + T D V 3 „	53 „	*Nil*	45 „	*Nil*
12-5-31	C V 3·5 „ + T D V 3·5 „	56 „	*Nil*	46 „	*Nil*
19-5-31	C V 4 „ + T D V 4 „	56 „	*Nil*	46 „	*Nil*
26-5-31	C V 4·5 „ + T D V 4·5 „	56 „	*Nil*	49 „	*Nil*
1-6-31	C V 5 „ + T D V 5 „	60 „	*Nil*	49 „	*Nil*
8-6-31	C V 5·5 „ + T D V 5·5 „	60 „	*Nil*	48 „	*Nil*
15-6-31	C V 6 „ + T D V 6 „	59 „	*Nil*	50 „	*Nil*
22-6-31	C V 6·5 „ + T D V 6·5 „	67 „	*Nil*	50 „	*Nil*
29-6-31	C V 7 „ + T D V 7 „	63 „	*Nil*	50 „	*Nil*
5-7-31	C V 7·5 „ + T D V 7·5 „	63 „	*Nil*	53 „	*Nil*
12-7-31	C V 8 „ + T D V 8 „	66 „	*Nil*	55 „	*Nil*
19-7-31	C V 8·5 „ + T D V 8·5 „	70 „	*Nil*	55 „	*Nil*
26-7-31	C V 9 „ + T D V 9 „	69 „	*Nil*	61 „	*Nil*
3-8-31	C V 10 „ + T D V 10 „	72 „	*Nil*	61 „	*Nil*
10-8-31	C V 12 „ + T D V 12 „	72 „	*Nil*	63 „	*Nil*
17-8-31	C V 15 „ + T D V 15 „	72 „	*Nil*	63 „	Small swelling No softening
28-8-31	C V 20 „ + T D V 20 „	72 „	*Nil*	69 „	Small hard nodules
3-9-31	C V 22·5 „ + T D V 22·5 „	72 „	*Nil*	60 „	*Nil*

Tested on 10-9-31

Goat A	Goat B
1 c.c. of serum neutralized 0·5 mg of pure cobra venom	1 c.c. of serum neutralized 0·6 mg of pure cobra venom
1 c.c. of serum neutralized 0·8 mg of pure dabora venom	1 c.c. of serum neutralized 0·8 mg of pure dabora venom

TABLE II

Showing the course of immunization of No 5 horse with cobra venom and treated daboia venom, anti-viperine potency of its serum before the commencement of immunization being less than 1 mg per c c

Date	Dose	Presence or absence of local reaction at the site of injection after three days	Neutralizing power of serum as tested on pigeons
9-1-32	C V 10 mg + T D V 10 mg + 1,500 units A T S	Small swelling which subsided gradually No pus formation	
20-1-32	C V 20 mg + T D V 20 mg + 1,500 units A T S	Small swelling No pus formation	
29-1-32	C V 40 mg + T D V 20 mg + 1,500 units A T S	Small swelling ½ oz pus evacuated	
8-2-32	C V 60 mg + T D V 60 mg + 1,500 units A T S	Small swelling ½ oz pus evacuated Photographed on 12-2-32	1 c c neutralized 0 5 mg of pure cobra venom and 1 c c neutralized 2 mg of pure daboia venom
18-2-32	C V 60 mg + untreated D V 60 mg (same brew as T D V) + 1,500 units A T S	Very big swelling (8 inches in diameter) photographed on 22-2-32 ½ lb of pus evacuated on incision Kept on discharging pus for four days	1 c c neutralized 0 5 mg of pure cobra venom and 1 c c neutralized 2 mg of pure daboia venom

N B—A T S = Anti tetanic serum

Since the technique of testing the potency of antivenin against daboia venom as described below has been introduced, we find that although our horses receive equal quantities of cobra and daboia venoms at each injection during their immunization the antigenic response for both the venoms is not by any means the

same. For instance, recently 1 c c of a horse's serum neutralized 2 mg of daboia venom but it did not neutralize 0 5 mg of cobra venom when tested in pigeons The reverse can be true also

We now test every sample of antivenin against both daboia and cobra venoms before we regard the animals as up to titre

For the above purpose it was desirable to fix up a standard of anti-viperine potency which must be satisfied by every brew of antivenin before it is passed for therapeutic use Kasauli antivenin in the past has been very favourably reported upon by clinicians all over India in the treatment of Russell's viper bites with toxæmia So we examined all the available brews in our stock to find out the minimum anti-viperine potency of our antivenins (*vide* Table IV) As daboia venom when injected intramuscularly into pigeons gives inconsistent results, we adopted the intravenous route attempted a few years ago by Anderson* for the determination of the minimum lethal dose (M L D) All the samples of daboia venom stocked here were examined, as a fixed M L D is essential for biological standardization The M L D 's of different samples tested on pigeons of 300 g weight are given below —

TABLE III.

Sample No 1 daboia venom	0 02 mg.
„ „ 2 „ „	0 03 „
„ „ 3 „ „	0 02 „
„ „ 4 „ „	0 03 „
„ „ 5 „ „	0 02 „

From the above results the M L D works out as 0 02 mg in standard pigeons Out of five samples examined, No 5 has been preserved in a desiccator and tested frequently for a full year It has constantly given an M L D of 0 02 mg in pigeons

For testing anti-viperine potency of Institute manufactured antivenin varying quantities of viper venom (usually 0 5 mg to 3 mg) dissolved in 1 c c of normal saline solution were added to 1 c c of the immune serum and the mixture after half an hour's incubation was injected intravenously in a set of pigeons while another set of three pigeons receiving 0 01, 0 02, 0 03 mg of venom alone, served as controls The neutralizing power of the serum was regarded as being equivalent to the largest dose of the venom which when mixed with serum failed to kill in 24 hours less the M L D of venom as determined by the control test

* Private communication to authors

The anti-viperine potencies of various brews of Kasauli antivenin as determined by the above technique are given in Table IV —

TABLE IV

Brew of antivenin	Neutralizing power against daboia venom	Brew of antivenin	Neutralizing power against daboia venom
V 1	2 mg	V 9	2 mg
V 2	1 „	V 10	1 „
V 3	2 „	V 11	2 „
V 4	1 „	V 12	1 „
V 5	2 „	V 13	1 „
V 6	1·5 „	V 14	1 „
V 7	2 „	V 15	1 „
V 8	2 „	V 16	1 „

From the results it is obvious that none of the sixteen brews tested had a potency less than 1 mg of daboia venom per c c of serum This we have adopted as a standard of anti-viperine potency of Kasauli antivenin

In test pigeons the death usually occurs in two to three minutes with unsteadiness followed by asphyxia Immediate autopsies showed no signs of intravascular clotting The death therefore appears to be due to action of daboia venom on the medullary centres and not to coagulation of blood in big vessels

Those who survive, unless the venom is quickly neutralized, develop unsteadiness but finally settle down In a fatal case it is invariably noted that the bird at first sways forwards and backwards, then throwing its head back falls down in a state of opisthotonos and dies subsequently with convulsions

DISCUSSION

From the above study it would appear that hepatic lipoids when allowed to remain in contact with daboia venom for a year at 37°C do certainly reduce its necrotic action on animal tissues Whereas in smaller doses there was complete absence of local reaction in horses, when the immunizing dose was increased to 60 mg a certain amount of necrosis though remarkably less than the one produced by untreated daboia venom was noticed Probably a longer contact with hepatic lipoids may detoxicate the venom completely Treated venom when dissolved in normal saline formed an amber-coloured solution and in spite of continuous shaking

with glass beads a certain amount of particulate matter remained in suspension These particles were probably cellular debris derived from the liver substance as the typical frothing of the solution pointed to the fact that the daboia venom had been dissolved

The local swelling after injection of treated venom, not going to the necrotic stage we attribute to the protein particles injected along with the treated venom If, however, as Vellard points out, the detoxication of viperine venom is due to lecithin which is abundant in liver tissue, it may be worthwhile trying the action of pure commercial lecithin on daboia venom under similar circumstances

Summary

1 The attenuation of the local necrotic effect of daboia venom by hepatic lipoids without interfering with its immunizing power has been confirmed

2 A biological test of ascertaining the anti-viperine potency of antivenins is described

3 The minimum anti-viperine potency of Kasauli antivenins is reported

Acknowledgment

We are deeply indebted to Colonel Sir S R Christophers, *Kt*, CIE, OBE, FRS, IMS, Director, Central Research Institute, Kasauli, for placing facilities at our disposal and for his sympathetic encouragement throughout these observations

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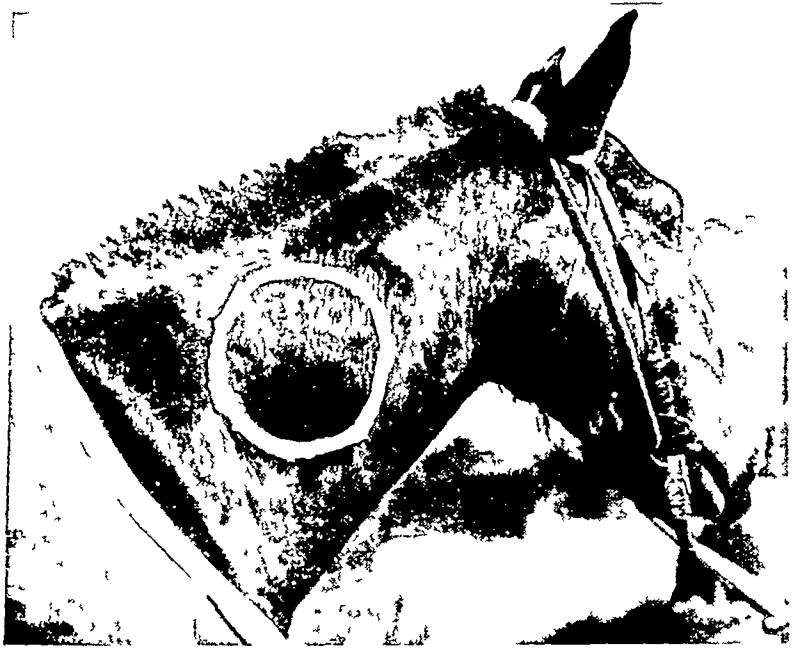
Plate IX

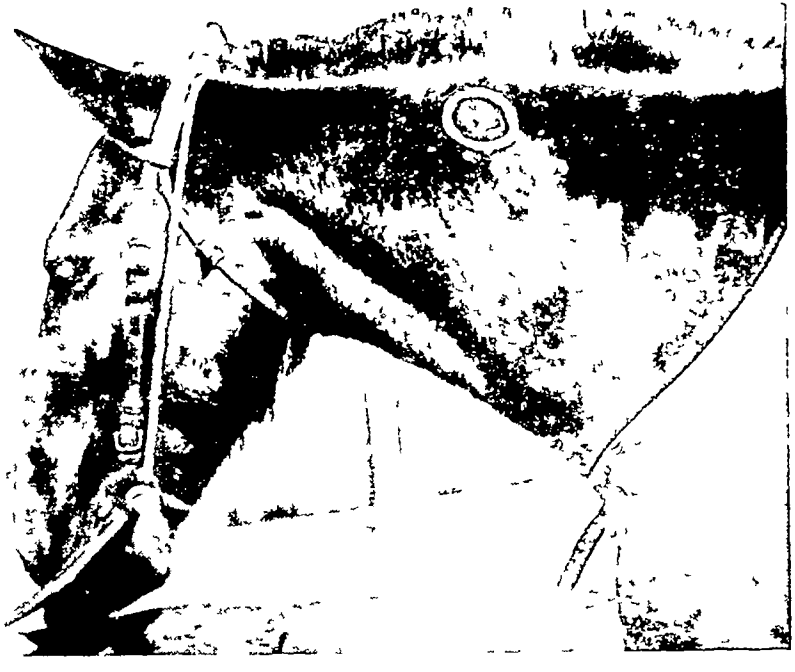
No 5 Horse



Photograph of 22 2 32

Showing inflammatory reaction (in white circle) about 8 inches in diameter after injection of untreated daboia venom combined with cobra venom (D V 60 mg + C V 60 mg)

No 5 Horse



Photograph of 12 2 32

Showing inflammatory reaction (in white circle) of about 2½ inches in diameter after injection of treated daboia venom combined with cobra venom (T D V 60 mg + C V 60 mg)

School and men of the Health Officer's section, Jullundur City, students at the Medical School Amritsar, men of No 3 Company Indian Hospital Corps, Lahore Cantonment and of the Health Officer's section Lahore City At the Lahore and Jullundur centres, I had an opportunity of examining persons coming from the North-West Frontier, from the hill areas of Kangra, Kashmir Garhwal, Nepal and Almora and from plains areas in the United Provinces In all one thousand persons were examined The blood material was taken during the hours of 7 and 10 at night and the thick films were treated with recognized methods of staining

I am grateful for the personal interest and kind help given to me during this investigation by the Superintendent of Police, Civil Surgeon, Head Master, Government School, and the Officer and Staff Ross Field Experimental Station for Malaria Research, Karnal, by the Deputy Commissioner, Head Master of the Normal School, and the Health Officer, Jullundur, by the Principal, Medical School, Amritsar, and by the O C, No 3 Company Indian Hospital Corps, and the Health Officer, Lahore

III. Results obtained.

A Physical aspects of the Punjab

The Punjab has been divided into four natural divisions in each of which the general meteorological conditions are believed to be fairly homogeneous These are (*i*) the Himalayan, with an average rainfall more, but nowhere less, than 36 inches, (*ii*) sub-Himalayan, with a rainfall 30 to 40 inches, (*iii*) the Indo-Gangetic plain west, having an average rainfall of 24 inches, and (*iv*) the south-west dry area, with extremely light and variable rainfall (*Imp Gaz, India*, 1908*g*)

The Punjab is subject to extreme vicissitudes of climate The temperature data given below have been taken from the records of the Meteorological Department, India, from 1921 to 1930, from the observations made at Lahore The temperature is expressed as mean of daily means

The range of variation between January and February is 50 2°F to 65 5°F, between March and April, 66 2°F to 87 7°F, between May and June, 81 8°F to 95 5°F, between July and August, 85 2°F to 92 0°F, between September and October, 74 4°F to 86 2°F and between November and December, 52 1°F to 65 3°F

The general level of the Punjab excluding the Himalayan division is between 1,000 to 1,500 feet above the sea-level (*Surveyor-General, India*, 1915) and wheat is the staple crop grown for sale (*Imp Gaz, India*, 1908*g*)

B Filariasis in the Punjab area

The blood of one thousand persons examined at night between 7 and 10 P M in the Punjab area showed uniformly negative results for filariasis

C Analysis of the results

(1) *Distribution of habitat of persons in relation to altitude*

0–500 feet, total 37, 500–1,000 feet, total 729, 1,000–2,500 feet, total 83, 2,500–5,000 feet, total 68 5,000–10,000 feet, total 83

(2) *Distribution of persons examined in relation to the district areas*

The Punjab area —Ambala, 6, Amritsar, 18 Cambellpur, 2, Delhi, 3, Ferozepore, 16, Gujranwalla 6, Gujrat, 23, Gurdaspur, 17, Hoshiarpur, 30, Jhelum, 9, Jhung, 5, Jullundur, 143, Kangra, 37, Karnal, 215, Lahore, 50, Ludhiana, 30, Lyallpur, 14, Mianwali, 9, Montgomery, 3, Multan, 7, Muzaffargarh, 6, Rohtak, 9, Shahapui, 13, Sialkot, 18, *Total* 689

The U P area —Almora, 47, Allahabad, 2, Budaun 2, Etawah, 1, Garhwal, 29, Gonda, 3, Hardoi, 2, Lucknow, 2, Meerut, 76, Muzaffarnagar, 45, Moradabad, 1, Pilibhit, 2, Rampur, 4, Saharanpur, 17, Sultanpur, 9, *Total* 242

The Kashmir area —26, *The Nepal area* —10, *The N-W F area* — Abbottabad, 1, Attock, 1, Bannu, 2, D I Khan, 3, Hazara, 8, Kohat, 1, Peshawar, 8, Rawalpindi, 9, *Total* 69

(3) *Distribution of persons in relation to age groups*

5–10 years, 6 persons, 10–15, 39, 15–20, 344, 20–25, 211, 25–30, 183, 30–35, 120, 35–40, 55, 40–45, 12, 45–50, 22, 50–55, 3, 55–60, 4, 65–70, 1

(4) *Distribution of persons in relation to class and type of population*

Hindus —Police, 33, School, 186, Military, 128, General, 44 *Mohammedans* —Police, 64, School, 149, Military, 65, General, 8 *Sikhs* —Police, 11, School, 48, General, 1 *Christians* — Police, 1, Military, 3, School, 1 *Depressed classes* —(Sanitary), 258

D *Culex fatigans*

No general survey of the mosquitoes was undertaken, but the data kindly supplied by Captain P J Barraud of the Malaria Survey of India (and to whom my thanks are due) point to the fact that *C fatigans* is one of the commonest mosquitoes of the Punjab

He reports as follows ' *Note on the distribution of* Culex fatigans *in the Punjab C fatigans* is one of the commonest mosquitoes of the Punjab and occurs in all the districts of that province It is a domestic species and is especially abundant in towns In such places as Amritsar, Lahore and Karnal, this mosquito is found in enormous numbers in the early part of the hot weather, and may be found swarming in such places as bathrooms It is common also in the Simla Hills up to elevations

of 6,000 feet or more The larvæ are found chiefly in domestic collections of water and often occur in places in which the water is foul though they have been found in abundance in such places as roadside ditches, stagnant water in drains, water butts, etc '

IV. Discussion of results.

The relationship between filariasis and certain physiographical and agricultural conditions of the terrain

During observations on filariasis in Bihar and Orissa, it was clearly shown that infection by *F bancrofti* varies with the nature of terrain It is highest in the sea-coast belt, high in the Gangetic plain (level 0–250 feet) and lowest in the sub montane arable areas Areas rich in rice cultivation appear to be the endemic centres (Korke, 1930*a*)

The physical aspects of Bihar and Orissa have already been described and here we may say that the conditions of temperature, humidity and certain other factors are expressed in the staple crop of rice (Korke, 1930 *a* and *b*)

In the Punjab these conditions are such that the staple crop is wheat The two areas differ profoundly in regard to the prevalence of filariasis In Bihar and Orissa it is the common condition In the Punjab examination of the night blood of one thousand persons did not yield a single positive result In both areas *C fatigans* is a common mosquito The most noticeable difference is in the level of the land and the nature of the staple crop

Acton and Sundar Rao (1931) have given a ' Map of India showing distribution of filarial infection based on examination of thick blood smears, (in which) the areas with filarial infection, areas with no filarial infection, and areas not surveyed ', are shown

The infected areas are widespread, and are shown extending from Assam to certain districts in the United Provinces Such areas also extend along the coastal regions of the Madras and the Bombay Presidencies When these areas are studied in the light of altitude and the staple crop, it will be seen that infection is continuous in the levels between 0–250 feet, and is usually restricted to 0–500 feet The staple crop in these areas is rice (*Imp Gaz , India,* 1908 *a* to *h*, and *Surveyor-General, India,* 1915)

An exception to the above observations is seen in a few isolated areas in the Central Provinces and on the Deccan plateau, like Bijapur and Bellary These areas are over 1,000 feet above sea-level

In the Central Provinces, rice is the most important crop in the province, being 24 per cent of the cropped area (*Imp Gaz , India,* 1908*d*) How far these endemic areas situated at a relative high altitude possess a high or low endemicity is not to be determined from the data supplied in the construction of the map

Without being in a position to specify what definite relation there may be between altitude and a certain agricultural type of land and the presence or absence of filariasis, the facts pointed out certainly seem to uphold some such relationship

V. Conclusions

1 Examination of the blood of one thousand persons taken at night between 7 and 10 P M failed to give any evidence of the incidence of filariasis in the Punjab

2 A comparison of conditions in different parts of Bihar and Orissa and the observations now recorded in the Punjab suggests that there may be some correlation between low altitude of a land and associated rice cultivation, and the occurrence of endemic filariasis

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ON THE VALUE OF WILSON AND BLAIR'S BISMUTH SULPHITE MEDIUM IN THE ISOLATION OF *B TYPHOSUS* FROM FÆCES AND SEWAGE

BY

LIEUT-COLONEL A D STEWART, MB, FRCSE, DPH, DTM, IMS,
Director, All-India Institute of Hygiene and Public Health, Calcutta,

AND

S C GHOSAL, MB, DPH,
Assistant Professor, School of Tropical Medicine and Hygiene, Calcutta

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BISMUTH sulphite medium was first introduced by Wilson and Blair in 1926 for the isolation of *B typhosus*

The rationale of this medium as explained by Wilson is as follows —

(1) *B typhosus* is able to reduce sulphite into sulphide in the presence of a fermentable carbohydrate Black colonies are thus produced on the medium

(2) Bismuth sulphite in the presence of excess of sodium sulphite suppresses the growth of most of the colon group of organisms

In 1927 they published some modifications and improvements on this medium, calling the modified medium 'Medium B' They claim to have got good results with this modified medium in the isolation of *B typhosus* from fæces and Belfast sewage Allison has obtained good results with this medium in the isolation of *B typhosus* from stools of patients and carriers Gray, Begbie and Gibson have also isolated *B paratyphosus B* from Edinburgh sewage with this medium

We had started work with medium B when another paper appeared in April 1931 in which Wilson and Blair suggested the addition of 2 c c of absolute alcohol to 100 c c of medium B The media finally developed are prepared in the following manner —

To 100 c c of melted 3 per cent agar are added 5 c c of a 20 per cent solution of glucose, 10 c c of a 40 per cent solution of sodium sulphite (anhydrous), and

5 c c of a standard bismuth solution After boiling for two minutes an addition is made of 1 gm exsiccated sodium phosphate dissolved in 10 c c of boiling water and 1 c c of 8 per cent solution of ferrous sulphate crystals To it is then added 0 5 c c of a 1 per cent watery solution of brilliant green and 2 c c of absolute alcohol

The standard *liquor bismuthi* is prepared by mixing 60 gm of bismuth citrate with 50 c c of distilled water and then 20 c c of *liquor ammoniæ* of specific gravity 0 880 and finally making the volume up to 500 c c with distilled water

It has been stated by some that the blackness of the colonies depends upon the brand of bismuth citrate used, but different brands that we have used have given equally good results in our hands

It should also be noted that the specific gravity of the *liquor ammoniæ* as advocated by Wilson and Blair should be strictly 0 880, any deviation from this gives indifferent results

Nature of colonies and the time that should elapse before they should be studied

In Wilson's opinion discrete colonies of *B typhosus* on the medium are uniformly black, small, flat and dry with a metallic lustre To gain experience of the nature of the colonies we inoculated this medium with different laboratory strains of *B typhosus* We did not get the uniform results observed by Wilson Some strains gave typical colonies but on many occasions the colonies differed in their characteristics and some strains reduced sulphite very poorly It should be mentioned that fresh strains should always be used in studying the colonies, as old laboratory strains give indifferent results

As regards the duration of incubation at 37°C, 40–48 hours' incubation is preferred With 24–30 hours' incubation, colonies were found to be too small and reduction of sulphite not marked enough for recognition

Isolation of B typhosus *from stools and comparison of results obtained with that of McKonkey's plates*

Twenty-five samples of stools were examined from patients giving positive Widal reactions With bismuth sulphite medium *B typhosus* was isolated 20 times, i e, 80 per cent positive, and with McKonkey's plates in 10 cases, i e, 40 per cent positive, when the findings were positive in both cases the number of *B typhosus* colonies was far greater in the bismuth sulphite medium

Isolation of B typhosus *from sewage of different sources, viz* —

(1) From septic tank effluents (both raw and filtered, before chlorination)

(2) From activated sludge plant

(3) From Calcutta sewers and main sewage outfall

Method of isolation

Fresh sewage in each experiment was collected in sterile bottles and brought to the laboratory Examination was always done within 4 hours of collection About a dozen plates were used in each experiment Each bottle was thoroughly shaken and each plate inoculated on the surface with about ½ c c of the sample and spread over carefully with an L-shaped rod The surface was allowed to dry and the plates inverted and kept in the incubator at 37°C After 40–48 hours' incubation suspected colonies were inoculated on a double sugar McKonkey's plate containing lactose and saccharose The non-fermenting colonies were tested for agglutinability with high titre serum If agglutination occurred the colony from which the bacilli was taken was subcultured on a nutrient agar slope After 24 hours, morphology, motility and the titre limit of agglutination were examined and different sugar tubes inoculated Only organisms conforming to all these tests of *B typhosus* were finally diagnosed as such

(1) *Isolation from septic tank effluents (both raw and filtered, before chlorination)*

Twenty-five samples of raw septic tank effluents were tested from different places Of these 20 gave positive results, i e , 80 per cent , we then examined whether the filtration process undergone by the raw effluent had any germicidal action on *B typhosus* Both raw and filtered effluents in same amounts were examined simultaneously from different plants 10 times *B typhosus* was isolated on 8 occasions both from the raw and the filtered and there was no marked difference in the number of colonies found in either sample showing that practically no destruction of *B typhosus* occurred by filtration

(2) *From activated sludge plant*

Both raw sewage and final effluents in one particular plant was examined for 7 consecutive days *B typhosus* was isolated 3 times (i e , about 45 per cent) from the raw but never from the final effluent, thus showing that *B typhosus* was destroyed in this purification process This is in agreement with our previous results

(3) *Incidence in Calcutta sewage*

The experiments carried out may be divided into two heads —

(*i*) Examination of different sewers of District No 1

(*ii*) Examination of sewage from main outfall

During the monsoon when Calcutta sewers are flooded with rain-water off and on we failed to isolate *B typhosus* from either source due to growth of a large number of reducing groups of colon organisms With the disappearance of the monsoon the coliform organisms also diminished in number and positive results began to be obtained

(i) *Examination of different sewers of District No* 1

Thirty samples were examined in all with 20 positive findings, i e, about 66 1 per cent positive

(ii) *Examination of sewage outfall*

Here our results were variable In November 10 samples were examined of which two were positive In December another 10 samples were examined with 3 positive findings

During this period we observed that on many occasions the plates were so much blackened that they were useless and also colonies in some were so crowded as to make discrimination difficult From January we inoculated only 4 small drops on each plate and discrimination of colonies was then possible In January and February 20 samples were examined of which 10, i e, 50 per cent, were positive

Viability of B typhosus *in sewage and stools*

Wilson and Blair recovered *B typhosus* from naturally infected Belfast sewage after 5 weeks when kept in a bottle in the laboratory We carried out similar experiments to find out the fate of *B typhosus* in naturally infected sewage in the tropics

Raw sewage giving a positive result was kept in a sterile bottle in the laboratory at room temperature and examined from day to day for the presence or absence of *B typhosus* Several experiments were carried out To start with we had in each experiment roughly 1 *B typhosus* per c c of sewage and when 10 c c of it were negative the result was considered to be negative We did not get the same results as obtained by Wilson and Blair The sewage gave negative results in 10 c c generally from 3 to 5 days and in all our experiments within 7 days The same results were obtained when sewage was artificially infected with about 1,000 *B typhosus* per c c

Viability of B typhosus *in stools*

Several samples of stool giving positive results were examined in the same manner as in the case of sewage In this case also we found that *B typhosus* disappeared in from 2–5 days, the longest period being 7 days

Conclusions

(1) We have found bismuth sulphite medium to be much superior to McKonkey's plates in isolating *B typhosus* from stools Out of 25 samples examined *B typhosus* was cultivated 20 times in bismuth sulphite medium whereas McKonkey's plates gave positive results only 10 times with the same material

(2) *B typhosus* was cultivated 20 times out of 25 samples of final septic tank effluents of different localities, showing that it is very common in septic tank effluents

It has also been shown that filtration of septic tank fluid has very little germicidal action on *B typhosus* whereas the activated sludge process has a definite germicidal action

(3) From the tributary sewers of Calcutta *B typhosus* can be readily cultivated Out of 30 samples examined from the sewers of District No 1, 20 proved positive At the beginning the number of positive findings was small in the case of main sewage outfall but in January and February out of 20 samples examined 10 were positive

(4) In our experiments *B typhosus* disappeared from sewage and stools on an average between 3 to 5 days, and always within 7 days Compared with temperate climates, the viability of *B typhosus* outside the body is much shorter in the tropics

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in the non-virulent forms, and which disappears from the virulent forms as they are 'degraded' by various means into the avirulent type. The importance of the carbohydrate fractions in pneumococcus immunity has been demonstrated by several workers. For example, Gaspari, Fleming, and Neill (1927) have shown that the loss of the specific carbohydrate of Type II pneumococcus involves the loss of the antigenic properties concerned in both active and passive protection of mice. They conclude that the antibodies to which protection is due are closely related to, if not identical with, the specific antibody to the carbohydrate fraction.

The question of the relation between virulence and carbohydrate fractions in the vibrios will be discussed below.

Material and methods

1. *Strains.*—Ten strains of vibrios have been used in this study. They were chosen to cover a wide range of source and characteristics (Table I), and include agglutinating vibrios from cholera cases (Ch 1, 2, and 4), a strain (Ch 1 A) which only differs from Ch 1 in being resistant to choleraphage Type A, and Ch 3 which is a rough strain from a cholera case. Three non-agglutinating water vibrios were also used (W 1, 2, and 3) and two non-agglutinating vibrios which were isolated from stools of cholera cases. No agglutinating vibrios were obtained from these stools.

As a control of the specificity of the precipitin cross-reactions, carbohydrate material was used which had been obtained from strains of *B typhosus* and *B dysenteriæ* Flexner.

These strains had been isolated at intervals ranging from a few weeks to three years before use. There is at present no evidence either for or against the view that prolonged laboratory cultivation may alter the character of the carbohydrate constituent. It is obvious that more information would be gained on this point, which is at present under closer investigation, by the use of cultures having widely different dates of isolation.

Table I also gives the agglutination titre of the agglutinable strains with standard Kasauli serum, the time necessary for coagulation to occur in the extraction process described below, and the roughness or smoothness as shown by the test with Millon's reagent (White, 1929).

2. *Method of extraction.*—The organisms were sown on Roux bottles, and incubated for 48 hours. The growth was then washed off in normal saline, and the solution brought to an acidity of N/20 with glacial acetic acid. Thirty bottles were dealt with as a unit, and the final volume of washings averaged 750 c.c. The bacterial mass was boiled on a sand bath under a reflux condenser until coagulation occurred (Table I). The coagulated mass was allowed to cool, usually in the ice-box overnight, and then run several times through a Sharples supercentrifuge until a semi-opaque brownish solution was obtained. This solution was precipitated with

TABLE I

List of strains of vibrio used in the present investigation

No	Origin	Approximate length of time since isolation	Titre of agglutination reaction with Kasauli antiserum	Coagulation time	Millon's test
Ch 1	Clinical cholera	18 months	1 2,500	6 hours	Smooth
Ch 1A*			1 500	5 hours	Smooth rough
Ch 2	Clinical cholera	5 months	1 1,000	12 hours	Rough
Ch 3	Clinical cholera	12 months	1 1,600	10 minutes	Rough
Ch 4	Clinical cholera	12 months	1 500	1 hour	Smooth
W 1	Water	6 months	Non agglutinable	3 hours	Smooth
W 2	Water	6 months	Non agglutinable	1 hour	Smooth
W 3	Water	3 years	Non agglutinable	7 hours	Smooth
X 1 †		1 month	Non agglutinable	2 hours	Rough
X 2		1 month	Non agglutinable	2 hours	Smooth

* This strain is similar to Ch 1, except that it is resistant to Type A choleraphage

† Strains X 1 and X 2 are non agglutinable organisms from cholera stools No agglutinating vibrios were found in these two stools

three volumes of 90 per cent alcohol, and placed in the ice-box overnight The heavy precipitate was pipetted off, separated from the alcohol as completely as possible by centrifuging, and taken up in 200 or 300 c c of water Insoluble matter was discarded and the solution again precipitated with alcohol As before, the precipitate was freed from any remaining insoluble matter and dissolved in 100 c c of water, where it formed a clear, brown-tinged solution, with a faint but unmistakable biuret reaction It was strongly acidified with glacial acetic acid, and boiled After cooling, the dark brown flocculum which had appeared was centrifuged off, and the supernatant fluid, which was now biuret negative, was precipitated with three volumes of alcohol The precipitate was dried, weighed, and dissolved in approximately N/20 NaOH to make a 1 per cent solution The yield from thirty bottles averaged 500 mg

A detailed chemical analysis of this material from the various strains listed in Table I is now being made It will be sufficient to indicate here that 1 per cent solutions of these substances give negative biuret Millon's, Hopkins-Cole, and xanthoprotein reactions They yield an intense purple colour with Molisch reagent, and the reaction is evident even at a dilution of 1 1,000,000 Boiling the solutions with dilute acids brings about the production of substances which reduce Fehling's reagent

3 *Technique of agglutination and precipitation tests*—The agglutination tests were carried out by Dreyer's technique After incubation at 56° for 2 hours the tubes were put in the ice-box overnight, and final readings then recorded Agglutinating sera were made to five of the strains, namely Ch 1, Ch 2, Ch 3, Ch 4 and W 1 The reactions of the strains to Kasauli serum were also obtained Rabbits were injected with increasing doses of killed vibrios at weekly intervals for three weeks, and the serum tested after an interval of 7 to 10 days

For the precipitation test 0 2 c c of the undiluted serum was added to an equal quantity of the 1 per cent solution of the extract After mixing, the tubes were incubated at 37° for two hours, placed in the ice-box overnight, and the readings recorded It was found that to obtain a satisfactory precipitating serum for these strains, intensive immunization must be used over a relatively long period The rabbits were given three injections of killed suspensions of the vibrios on alternate days, and then allowed to rest for a week The injections were then repeated The number of vibrios in each dose was gradually increased, during the injections, from approximately 500 million to 3,000 million, and the period that elapsed before a satisfactory titre was reached was usually twelve weeks The precipitin titres for the homologous carbohydrate extracts at the end of the immunization period lay between 1 100 000 and 1 500,000, in one case only (W 1) was a titre of 1 1,000,000 reached When these sera were used in the agglutination test the results were the same, with non-agglutinating organisms, as when sera from less intensively immunized animals were used

Results

The agglutination and precipitation cross-reactions between the various strains and antisera are set forth in Table II

With two exceptions (Ch 3 antiserum with strains Ch 2 and Ch 4), which will be mentioned later, all of the Ch antisera and the Kasauli antiserum agglutinate all of the vibrios from cholera cases, and none of the other vibrios The antiserum to the water vibrio (W 1) agglutinates only its homologous organism and W 2 The Flexner and typhoid strains are not, of course, agglutinated by any of these antisera

TABLE II

A comparison between agglutination reactions (whole organisms) and precipitation reactions (carbohydrate extracts) in agglutinating and non-agglutinating strains of vibrio

Strains and Extracts	ANTISERA											
	Ch 1		Ch 2		Ch 3		Ch 4		W 1		Kasauli	
	Agg	Precip	Agg	Precip	Agg	Precip	Agg	Precip	Agg	Precip	Agg	Precip
Ch 1	+	++++	+	++	+	+++	+	+++	0	+	+	0
Ch 1A	+	++	+	++	+	++	+	++	0	++	+	0
Ch 2	+	+++	+	+++	0	++	+	++	0	+++	+	0
Ch 3	+	±	+	0	+	+	+	+	0	0	+	0
Ch 4	+	++++	+	++++	0	+	+	++++	0	++++	+	++++
W 1	0	++++	0	+++	0	+++	0	+	+	++++	0	+
W 2	0	++	0	+++	0	+	0	+	+	++	0	++
W 3	0	+++	0	+++	0	+++	0	+	0	+++	0	0
X 1	0	+	0	++	0	±	0	+	0	+++	0	0
X 2	0	+++	0	+	0	+++	0	+	0	+	0	+++
Flexner	0	0		0	0	0		0	0	0		0
Typhoid	0	0		0	0	0		0	0	0		0

The agglutination reactions are indicated simply as positive (+), or negative (0) The precipitin reactions are indicated as follows ++++, complete clearing, +++, heavy flocculum, incomplete clearing, ++, light flocculum, no clearing, +, flocculum visible with hand lens, ±, doubtful, 0, negative

Turning to the results of the precipitin tests, it is at once obvious that the use of the carbohydrate fraction gives quite a different picture of the relation between the groups of vibrios The table shows in general that cross-reactions are present which run through the three groups (Ch, W, and X) and that these cross-reactions are obtained with all of the sera used The carbohydrate fractions, then, if not identical, are at least closely related in the agglutinating and non-agglutinating organisms

Since Kasauli antiserum was used as a standard antiserum to test the agglutinability of the various organisms its reaction in the precipitation test was also tried As the table shows, it precipitates with the carbohydrate of one of the agglutinating organisms, and of three of the non-agglutinating strains A negative precipitin test with this antiserum is not informative, since it is primarily an agglutinating serum The positive results, however, are important in showing further that cross-reactions between the carbohydrates of non-agglutinating strains and antisera of agglutinating strains do occur

Considering now the exceptions to the general statements which have been made, it is interesting to note that all of these exceptions relate to strain Ch 3 or its antiserum This strain is markedly different to the other strains used It is extremely rough in its growth, forming closely knit masses of organisms which are removed from the surface of the agar with difficulty On boiling it coagulates at once, and the yield of carbohydrate is only about one-half as much as with the smooth organisms As shown in the table, the cross-reactions of Ch 3 carbohydrate are doubtful with antiserum Ch 1, negative with antisera Ch 2 and W 1, and weak with the other two antisera Its precipitating antiserum gives only a doubtful reaction with the extract of X 1, and its agglutinating serum fails to react with strains Ch 2 and Ch 4

A detailed investigation of the carbohydrates from this and other rough strains is at present in progress

The extract of strain Ch 1 A, which is strain Ch 1 made resistant to Type A choleraphage, does not differ from the extract of the parent strain in its reactions to the various antisera This resistant strain has tended to acquire some of the properties of roughness, as shown by its reaction to Millon's reagent, and by the type of growth it exhibits It is far, however, from being a rough organism of the type of Ch 3

None of the antisera reacts with the carbohydrates derived from the Flexner and typhoid strains, showing that at least in respect to these two organisms we are dealing with a carbohydrate specific to the vibrios

Discussion

From the evidence which has been presented it is clear that these strains of vibrio, with the possible exception of the rough strain, contain a common or closely

related precipitable carbohydrate fraction Homologous and heterologous antisera precipitate these substances, but do not precipitate similar fractions from *B dysenteriæ* Flexner or *B typhosus* The question of the relationship between the fractions from the different strains will, it is hoped, be settled by the chemical analysis now under way

That serologically distinct groups of vibrios should have non-protein fractions which precipitate with heterologous antisera recalls the work of Hitchcock (1924), in which he showed that immunologically distinct groups of hæmolytic streptococci also yielded substances which were non-type specific and gave cross-reactions Lancefield (1928*a*) found that this species specific substance was probably a carbohydrate

It is probable that we are dealing here with a non-type specific fraction of *Vibrio* Whether this carbohydrate is species specific, it is of course impossible yet to say, since there is no accepted criterion for determining the characteristics which differentiate the various species of the genus *Vibrio* If the case is analogous to that of *Streptococcus hæmolyticus*, the agglutinable and non-agglutinable forms of *Vibrio* would rank, on the basis of the present findings, as types of a single species

When an antiserum to an agglutinating organism does not react with a non-agglutinating organism, but does, as in the present case, react with a fraction of the same organism, it is obvious that this fraction cannot be responsible for the non-agglutinability It is possible that this difference will be found to depend not on the difference of proteins in the two groups, but on the different antigenic complexes of protein plus carbohydrate in the agglutinating and non-agglutinating vibrios

In respect to a possible relationship between virulence and carbohydrate content in the vibrios, analogous to that found in the pneumococci, it is clear that the truth must be found in one of the following categories

1 The carbohydrate, as in pneumococcus, is present in virulent organisms, and is absent in the non-virulent forms

2 The carbohydrate of the genus *Vibrio* has no connection with virulence, and this characteristic may be connected with some other factor, similar perhaps to the protein-like M factor of virulent hæmolytic streptococci, described by Lancefield (1928*b*)

3 Virulence in the genus *Vibrio* differs from virulence in some other organisms which have been investigated by having no connection with any particular cellular constituent, but is a function or result of the activity of the cell as a whole

Further work is needed to determine which of these possibilities will prove correct for the genus *Vibrio*, or for *Vibrio choleræ*, if indeed such an entity exists as a species

SUMMARY.

The serological reactions of a precipitable, non-type specific, carbohydrate-like fraction isolated from agglutinating non-agglutinating, rough, and smooth strains of *Vibrio* are described The possible relationships of this substance to virulence, and its analogies with similar substances from other organisms are discussed

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CULTIVATION OF VARIOUS LEISHMANIA PARASITES ON SOLID MEDIUM

BY

J C RAY, M D,
In-Charge, Protozoological Survey,
Central Research Institute, Kasauli

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I INTRODUCTION

IN 1881 Robert Koch first introduced the solid culture media in bacteriology Since then bacteriologists have incessantly studied the requirements of bacterial growth and developed an almost perfect technique for their cultivation This alone made possible the study of morphology, biology, chemistry, antigenic properties and structure of the bacterial body Compared to this achievement in bacteriology, little is known about protozoa This has been chiefly due to the inability to grow protozoa in a satisfactory manner Of true phylogenetic relation there is none whatsoever between a bacterium and a protozoon, the former is a plant as much as the latter is a unicellular animal organism The parasitisms of both groups of disease-producing microbial organisms have, however, one factor in common—that they have to live and multiply on the resources of the host's body Judging from this side, while the bacterial cultivation has been so successful, cultivation of true protozoal parasites should not be an impossible task It is natural to expect that success will depend largely on ingenious methods of preparation of media and their presentation to parasites must correspond to the physical conditions in which they usually survive and multiply It will mainly be a question of overcoming technical difficulties

In the cultivation of protozoa we have to consider (1) their complicated life-cycle, (2) the complex nature of the body structure, (3) the varied methods of nutrition, and (4) marked features of their selective organ or organs of location in particular susceptible host bodies The writer's experience of various blood flagellates shows that they are capable of fairly rapid multiplication, if only they

are grown on suitable media under optimum conditions Unlike bacteria the rate of multiplication of a protozoon is much slower and so also unlike bacteria its multiplication is continuous under favourable conditions It appears that the logarithmic phase of growth is a fairly prolonged one, this becomes evident from the heavy growth of parasites capable of being produced and from comparatively few dead parasites met with when examined under the microscope

Although the life of a protozoon is a complex biological process yet its demands in artificial cultivation have often been found to be remarkably simple This is true with free living protozoa and the saprophytes the pathogenic protozoa on the other hand require blood as an essential constituent in the medium and are much more exacting in their food requirements for growth The following observation made as regards the food requirements of Leishmania parasites might prove of some help to the cultivation of other protozoal parasites It has been found that glucose is an important source of energy to the parasites and inclusion of glucose in culture media is essential to obtain satisfactory and rapid growth Blood containing complex proteins is evidently the most significant requirement for animal parasites It has been found that the serum supplies the nitrogenous organic food substances and the constituent of the red cell acts as a stimulus to growth, neither serum nor hæmoglobin alone produces good growth It has also been noticed that inclusion of simple protein and protein derivatives further increase the efficiency of the media It is an interesting fact to note that pathogenic parasites require a wide variety of protein in the media In bacterial cultivation mineral salts play a trivial part and little is known about it In cultivation of blood flagellates the writer has noticed the importance of various mineral salts Greater care should therefore be taken in selection of salts in protozoal cultivation It seems these organisms grow better under partial oxygen pressure In preparation of culture media for any protozoon it is advisable to use the blood of a susceptible animal and great care is to be taken to embody in it the nearest approach to the natural conditions, namely, the sites of location of the parasites in host's body, the physical conditions that prevail there and the method adopted by the parasites to take their nutrition from the host's body

Considering all these factors and by devising new and suitable techniques, it is expected that protozoal cultivation can be brought to a successful issue

II Résumé of previous works on cultivation of Leishmania parasites on solid media

The successful cultivation of blood and insect-flagellates on the surface of glucose-blood-agar plates was first carried out by Noller in 1917 It was by a simple ingenious method that he was able to cultivate with considerable success

L ctenocephali, *L fasciculata*, *T melophagium*, *L donovani*, and various other trypanosomes The medium he used consisted of—

Agar-agar	10 g
Grape sugar	10 ,,
Slightly alkaline horse flesh bouillon	1,000 c c

These were heated in boiling water sufficiently long to dissolve the agar and kept standing overnight Next day the agar mixture was again melted, filtered through cotton-wool and passed into sterile tubes, 12 to 18 c c in each tube The tubes were then sterilized in a steamer for 45 minutes on two successive days and stored in a cold place Before use the agar mixture in test-tubes was melted and at a temperature of 50°C , an equal quantity of defibrinated horse's blood was added to it and the blood and agar were then thoroughly mixed and poured into special Petri dishes (10 cm by 2 cm) The plates were then set in an ice-chest and inoculated as soon as set They were then incubated at 22°C upside down and, to prevent contamination and drying, a small quantity of 0 02 per cent corrosive sublimate solution was constantly maintained on the bottom of the lid

Noller obtained good growth of *Leishmania donovani* on the sixteenth day after the inoculating material had been taken from the NNN medium and the tenth day after inoculation had been carried out from one plate to another

By following Noller's technique Mayer and Ray (1928) grew various Leishmania parasites on a somewhat modified Noller's medium The following is the medium used by us —

Agar-agar	30 g
Grape sugar	20 ,,
Slightly alkaline Liebig's broth	1,000 c c

We were not only successful in growing various Leishmania parasites but at the same time observed that various species of Leishmania produced different types of growth on the surface of the medium *Leishmania donovani* grows on the streak without true lateral outgrowth—*Leishmania tropica* gives luxuriant growth and shows three distinct zones, a thick middle zone surrounded by a thinner zone from which extend parallel outgrowths—*Leishmania brasiliensis* grows in a thin surface film without any outgrowths from the margin The rate of growth among various species was also observed to differ considerably These different types of growth can be explained on the basis of the mode of progression, general structure of cytoplasm and rate of sedimentation of individual types of Leishmania parasites

Although much satisfaction was derived at the successful cultivation of various Leishmania parasites, specially because they are the agents of a number of diseases that prevail all over the world, the author was not satisfied at the slow rate of multiplication noticed on the plates from day to day , heavy growths were not

available before 10 to 12 days and characteristics in types of growth could not be ascertained before 15 days Apart from disadvantages that such a prolonged cultivation would cause to research workers and in preparation of vaccine, there are other serious objections The longer the media is kept in the incubator, the less becomes its efficiency As the oxyhæmoglobin changes, the medium gradually takes on a chocolate colour due to methæmoglobin which has a greatly diminished power in carrying oxygen Secondly, as the parasites grow certain toxic products of their metabolism are formed which, if allowed to accumulate by too prolonged a cultivation would cause injury and alterations to the growing parasites Thirdly, a certain amount of hæmolysis of the medium takes place, owing either to enzymes or acids or toxic products produced during the process of protozoal multiplication which makes it impossible to obtain pure cultures free from blood and extraneous matters

Since 1928, the author has been trying to produce a medium on which a luxuriant growth of these flagellates by more rapid multiplication within a few days could be made possible After all, artificial cultivation on a synthetic medium has one advantage, namely, the parasites are not subjected here to the adverse influences of the defensive mechanism of the host's body Considering the delicate structure of Leishmania parasites which is so much exposed to external influences for want of a protecting membrane and the simple method of osmosis that they adopt in getting their food material, I could, however, think of no other better technique than the one advanced by Noller My attention was therefore chiefly directed to improve the culture medium in order to supply all the food requirements and to determine the optimum rate of growth produced by each of the constituents Further, great care was taken in preparation of the medium so that it might fulfil the many-sided requirements of an ideal medium For the sake of economy in culture medium and work, the cultivation of these parasites has also been tried in test-tubes, after a great many experiments with test-tubes of various shapes, I have been able to devise a special type of test-tube which is extremely simple to work with and never fails to give full satisfaction As the parasites multiply the growth appears initially transparent, glistening like a dewdrop, then gives a whitish translucent appearance which passes over to almost white, then to a greyish white massive growth

III Preliminary notes on preparation of a solid culture medium

The essential characteristics of a solid medium are its adequate consistency and inherent quality of high grade diffusibility Agar-agar, the seaweed-gel, imparts the desired solidity, durability and resistance to the medium and, as it has the property of absorbing a large quantity of water by molecular imbibition, it serves the purpose of a culture medium admirably The efficiency of solid medium

for cultivation of flagellates will greatly be enhanced if, along with other food requirements, an abundance of water in physico-chemical union can be incorporated into it The flagellates growing on the surface of the medium must have the constant supply of fluid as they are subjected to rapid evaporation for want of an adequate protecting membrane enclosing the protoplasmic content Water is the essential constituent of protoplasm and without this, it cannot function Besides it functions as a good heat absorbant, thus acting as a buffer by taking and giving up heat generated by the process of protoplasmic metabolism, it has another remarkable property from the point of view of the preparations of a medium Water dissolves all the substances that the organisms require for nutrition On the other hand solid agar medium has essentially the properties of a gel, it consists of two distinct phases one solid and the other liquid As it contracts due to pressure exerted by surface tension, the liquid food-stuff is squeezed out on to the surface of the medium, when the plates are kept upside down, and thus becomes available to flagellates

It is clear from this that agar-agar, besides lending support to culture medium, has other physical properties which are of great importance It has been suggested that an interchange of reactions takes place between the medium and organisms that grow on it, the agar-agar giving up the food-stuff due to its property of contraction and taking in by absorption the waste products of organism This is perhaps one of the reasons for the rapid multiplication of flagellates that takes place on the solid medium

IV Preliminary experiments on preparation of the culture medium

In order to achieve success, two factors are to be followed in preparation of a culture medium, namely, composition of nutrient material and the physical conditions under which the medium is to be offered to the parasites

A *Physical condition comprising the consistency and diffusibility of the medium*

(1) Powdered agar was found to be useless, even as much as 4 per cent agar failed to give adequate consistency to the medium, apparently it has a greatly diminished power of absorbing water and holding it in physical combination within it In preparation of the medium other agar-agar (fibres) must be used

(2) To determine the most suitable percentage of agar which will lend adequate support to the medium against the sheering stress of a platinum loop and allow the subsequent washing of the culture from the surface without admixture of blood and extraneous matter, the mediums consisting of 1, 1 5, 2, 2 5, 3, 3 5 and 4 per cent agar were prepared and at 55°C varying proportions of defibrinated horse blood was added, thoroughly mixed and poured into Petri dishes They were allowed to

set at room temperature for one hour and then inoculated; it was found that the best all-round result was obtained by 2 5 per cent agar

(3) To achieve the maximum swelling of the agar and increase in viscosity of the medium the following procedure in preparation of the medium is of importance The agar-agar should be cut into small and thin pieces in order to allow a larger surface for rapid and maximum absorption of water They should then be allowed to soak in distilled water overnight at a sufficiently low temperature resulting in uniform swelling and hydration of the submicroscopic solid aggregates

(4) Clearing the agar solution with egg white and filtering it through cotton-wool have proved unnecessary, as they tend to soften the medium and deprive it of its original consistency

(5) It has been mentioned that agar-agar absorbs water and holds it in a loose physical or physico-chemical combination within it When the agar medium is sterilized in the autoclave at 120°C under pressure of 15 lb it has been found to lose its hydrophilic property, i e, attraction for water to a considerable extent The water of condensation oozes out of the medium freely when plates are inverted and within a comparatively short time the medium turns out to be dry and unsuitable for rapid multiplication of flagellates The mixing of the ingredients of the medium should be carried out in a water bath and then sterilized in a steamer on two successive days

B *Notes on the experiments regarding the composition of the culture medium*

It is not possible here to give in detail the numerous experiments undertaken to find out the suitable composition of various ingredients incorporated into the medium The following are to be regarded as mere conclusions drawn from the experiments A constant ratio of defibrinated horse blood was added to the medium in proportion 1 5 each time

1 The addition of glucose greatly increased the efficiency of the medium, the growth was visibly better It was found that the best growth was obtained with the medium containing 2 per cent glucose

An important observation was made as regards the influences of various sugars on the rate of growth among the different species of Leishmania parasites *L donovani* and *L infantum* were found to be in a position to utilize all the sugars, namely glucose, mannite, lactose, saccharose, salicin, maltose and dulcite, though the rate of growth was more marked in glucose, *L brasiliensis* and *L tropica* grew very well in glucose, the former could utilize lactose saccharose and maltose only to a very limited extent and the latter was not at all touched by other sugars

2 An improvement was obtained by inclusion of 2 per cent peptone (Witte) into the medium

3 Further improvement was observed when beef-infusion or 2 per cent meat extracts were added to the medium Of the two, the beef-infusion proved decidedly superior to Lemco broth, apparently due to its additional nutritive elements which are destroyed by exceedingly high temperature used in preparation of the extracts The beef-infusion is to be preferred for another reason It gives a clearer colour to the basic medium and with admixture of blood in it, imparts a brighter red colouration to the medium It thus offers an excellent contrast to the growing flagellates and enables one to form a judgment as to the quality of a medium by observing rapid growth of the parasites from day to day

4 It has been observed that an addition of one per cent asparagin, the acid amide, to the medium further improved its quality, in particular *L brasilhensis* and *L tropica* grew remarkably well when this was added to the medium

5 The quality of the proposed medium was enhanced by addition of various salts to it It is interesting to note that chemically pure salts accelerate the growth The salts that are found in infusion or in blood are bound up with organic matter It is probable that salts alter the physical condition of the nutritive fluid in such a way that they act as a stimulant to the parasites

The following combination of salts in quantities specified against their name has been found to give the most satisfactory growth —

	Per cent
Sodium chloride	0 6
Potassium phosphate	0 05
Magnesium sulphate	0 05
Lithium chloride	0 02
Calcium chloride	0 02

6 0 5 per cent glycerine was added to the medium in order to give a slight emulsified colloidal character to the medium as a precaution against rapid evaporation It was observed that it gave no nutritive contribution to the growth of Leishmania parasites

7 The most characteristic feature of Leishmania parasites is their inability to grow in any medium however ingeniously prepared unless blood is incorporated into it

(*i*) It has been observed that the optimum percentage of blood required for achieving the best growth falls within a definite range and bears a direct relation to the percentage of agar embodied into the basic medium

(*ii*) Human blood and blood taken from various animals were then examined to find out their effect on growth It was found that human blood gave the best result, the rabbit's blood came next to it, bloods from guinea-pig and horse proved equally good Sheep's blood produced also good growth though it proved inferior to rabbit's blood It was found that the parasites grew well in the presence of

all the bloods mentioned here without any marked difference For routine work, bloods either from rabbit or from horse are to be preferred from the point of view of growth as well as other reasons

(*m*) Next the blood was variously prepared and examined to find out which of the constituents of blood promote growth Table below indicates the blood variously prepared and their influence on growth of different Leishmania parasites

TABLE

Showing the effect of variously prepared blood on growth

Number	Blood		Rate of growth			
			L tropica	*L brasiliensis*	*L donovani*	*L infantum*
1	Whole blood	Citrated	E	G	G	G
		Non citrated	E	E	E	E
2	Defibrinized blood		E	E	E	E
3	Pure serum free from red cells		P	N G	N G	N G
4	Washed red cells free from serum		F	P	N G	N G
5	Hæmolysed blood	Row's saline hæmoglobinized solution	G	G	G	G
		By thawing and freezing	E	E	G	G

E = Excellent G = Good F = Fair P = Poor N G = No growth

It will be noticed from the above table that the whole blood is required for growth—neither serum nor red cells alone constitutes the food requirements for the growth of these flagellates Further hæmolysed blood does not produce as good a growth as the whole blood does Hence the whole blood should be used for purpose of cultivation

C *Hydrogen ion concentration on growth*

The flagellates were found to grow in every hydrogen ion concentration of the medium—but the optimum growth was noticed between pH 6 2 to 8 As a

routine the pH of the medium was adjusted to 7 4 in order to have uniformity in the medium

D *Oxygen requirements for growth*

The anaerobic cultivation was tried and it was found that the flagellates do not multiply in absence of free oxygen—although they can survive as long as seven days The best growth was obtained under partial oxygen pressure

E *Influence of temperature on growth*

The optimum temperature for growth was found to be 22°C—a higher temperature was found to be decidedly harmful for growth of the flagellates on solid medium

V The preparation of the solid medium on the basis of preliminary experiments

Constituents

(1) Dist water	600 c c
(2) Agar-agar (fibre)	25 g
(3) Glucose	20 ,
(4) Peptone (Witte)	20 ,,
(5) Asparagin	10 ,,
(6) *Salts*	
Sodium chloride	6 ,,
Potassium phosphate	0 5 ,,
Magnesium sulphate	0 5 ,,
Calcium chloride	0 2 ,,
Lithium chloride	0 2 ,,
(7) Glycerine	5 c c
(8) Beef-infusion	400 c c

Method of preparation of the basic medium

(*a*) Cut 25 g of agar-agar (2) in small and thin pieces, place them with 500 c c distilled water (1) in a wide sterilized flask, shake vigorously to emulsify and leave the flask standing overnight at a low temperature

(*b*) Preparation of beef-infusion (8) Add one pound of finely chopped lean beef, free from fat or tendons, to 500 c c of water and allow to stand overnight in a cool place Next day boil the infusion for an hour, strain through a coarse towel and squeeze out the residue and filter

To 400 c c of this infusion add (4), (5) and (6) and dissolve by heating it in a water-bath, then sterilize in autoclave for 15 minutes

(*c*) Dissolve 20 g of glucose (3) in 100 c c of distilled water and keep ready for use

(*d*) Pour (*b*) and (*c*) into the flask containing agar-agar, dissolve the whole in a water-bath by using a sufficiently powerful flame* , if difficulty is encountered, bubble steam into it for a while, say 10 minutes by connecting a glass tube dipping right into the medium from an adjacent flask with water boiling in it When completely dissolved add 15 c c glycerine (7) to it

(*e*) Adjust the pH at 7 4

(*f*) Distribute while hot in measured quantities into small flasks or tubes sterilized beforehand

(*g*) Sterilize them in Koch's steamer 45 minutes on two successive days Autoclave sterilization under pressure should always be avoided

(*h*) The basic medium is then ready for use or it can be stored in a refrigerator for quite a long time It is a firm gel which vibrates when the containing vessel is tapped To prevent evaporation, the flasks and tubes are better sealed with collodion caps, introduced by Fried (1928)

VI Preparation of plates for cultivation

1 The following sizes of plates are used by me and approximate quantities of medium required for satisfactory cultivation of flagellates are specified below —

Plates	Dish	Lid	Quantities of approximate media
Large size	10 cm × 3 cm high	11 cm × 1½ cm high	30 c c
Medium ,,	8 cm × 3 cm ,,	9 cm × 1½ cm ,,	25 c c
Small ,,	6 cm × 2½ cm ,,	7 cm × 1½ cm ,,	15 c c

2 When required, melt the basic agar medium in water-bath and at 55°C add defibrinated blood in the proportion of one part of blood to five parts of basic medium Mix thoroughly and pour into Petri dishes and allow to set for an hour or two

3 When set, the plates are turned upside down and inoculated with a slightly bent loop from below

The following precautions are to be followed when inoculating a plate Place a filter paper on the table and moisten it with corrosive sublimate solution, remove

* At high altitudes like that of Kasauli (6,000 ft) difficulty in melting the gel is experienced due to the boiling point being low

the inner dish from the lid and place on the wet filter-paper, fill up the lid with a small quantity of corrosive sublimate solution (strength 2 g 1,000 c c)—then hold the lid in a slanting position with the left hand, quickly lift the dish with the right, place slowly at first with one edge just dipping in the solution and then the whole of it whilst turning the left hand to level the lid, in order to allow the corrosive sublimate to flow all over the bottom Every care should be taken that the corrosive solution does not sprinkle on the surface of the medium

4 Incubate at 22°C Add fresh corrosive sublimate solution when it has dried up

5 If contamination occurs in any portion of the plate, cut out the spot from below with a knife previously sterilized in the flame

6 Heavy and luxuriant growth is obtained in 4 to 5 days For experimental work wash the culture with saline and transfer it into a test-tube

VII Cultivation in test-tubes

Recently Row (1930) grew Leishmania and allied flagellates in wide test-tubes containing equal quantities of 3 per cent saline agar and hæmoglobinized saline solution In the usual way slopes were made and allowed to remain undisturbed overnight The water of condensation was then drained to the bottom by keeping the tubes in an upright position, first at 37°C for some hours and then in the ice-box The condensation fluid was then removed carefully with a pipette and kept sterile for subsequent use Inoculation with a drop or two of culture was made at some distance from the bottom, after which 1 or 2 c c of condensation fluid were introduced into the tube, care being taken not to moisten the surface of the medium The tubes were then incubated in the slanting position with the surface downwards

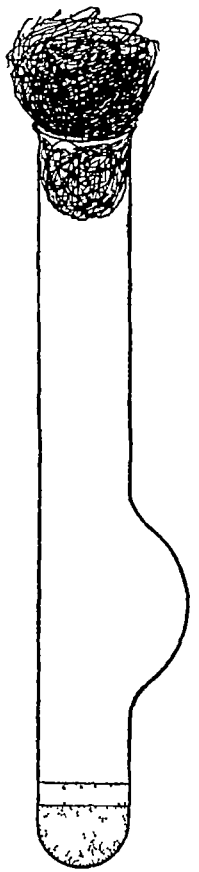

Fig 1 —Special form of test tube for test tube cultivation of flagellates

The method, followed by the author, is to utilize the condensation fluid as far and as long as possible Fig 1 shows the special form of test-tube used by me for test-tube cultivation of flagellates One side of a wide test-tube has been blown in such a way as to form an oval flat cup of about 3 c c capacity The test-tube contains initially 2 c c of the basic medium, when required it is melted and at 55°C to this is added 1 c c of defibrinated blood and thoroughly mixed The tube is then held in an inclined position so that the fluid collects in the oval cup The tube is then placed on a suitable stand in the horizontal position as shown in

Fig 2 and left undisturbed for 18 to 21 hours After this period it is inoculated and incubated, the test-tube being kept always horizontal with the surface of the medium uppermost

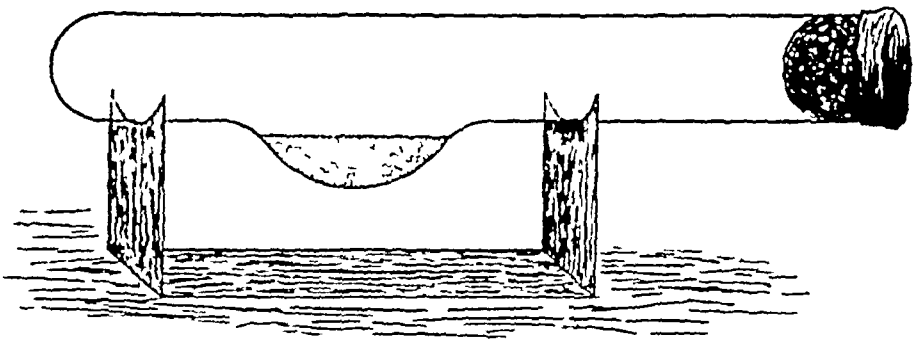

Fig 2 —Horizontal position of the tube on a suitable stand

The fluid oozing out of the medium initially collects at the bottom of the cup, but the weight of the medium displaces it subsequently so that ultimately it rises to the surface

It has been found necessary that the level of the culture medium should always remain a little below the dotted line, as shown in Fig 3 If it reaches the dotted line the condensation fluid does not collect on the surface of the medium, with the consequence that it will dry up soon

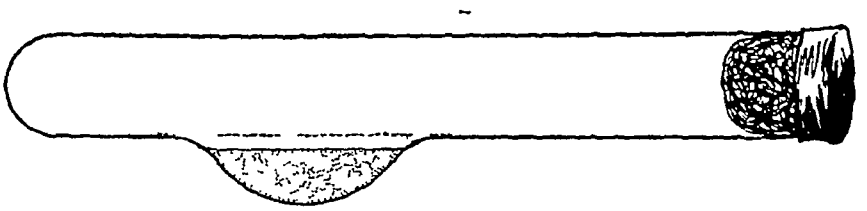

Fig 3 —Level of the surface of the medium should be slightly below the dotted line

By following this procedure beautiful growth is obtained within a week

Recently the author has tried to cultivate the flagellates on slopes in ordinary test-tubes (Fig 4) To 8 c c of the basic medium, 4 c c of defibrinated blood was added and slopes, $2\frac{1}{2}$ inches long, were made in test-tubes (one inch diameter) The tubes were then inoculated and incubated in a slanting position (the cotton-wool end lower), with the surface downward The tubes were never held in

an upright position By this method also abundant growth was obtained within a week

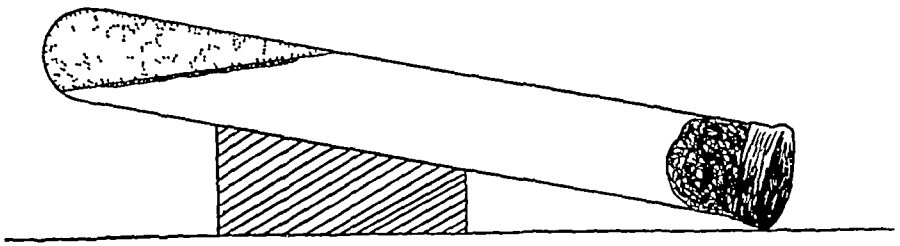

Fig 4 —Cultivation of flagellates in ordinary test tube

Conclusions

The attempt to obtain an abundant growth of Leishmania parasites in a comparatively short time has met with considerable success The author has developed two methods, the plate culture and the test-tube culture methods, both of which have proved very satisfactory

Acknowledgments

I take this opportunity of expressing my most grateful thanks to Prof J G Thomson for offering me excellent laboratory facilities and for his kind interest in the work, the greater part of which was completed in his laboratory at the London School of Hygiene and Tropical Medicine I would also like to thank Mr W Cooper for his willing assistance

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A SUITABLE FORMULA OF RINGER'S SOLUTION FOR PERFUSION WORK ON THE HEARTS OF INDIAN FROGS *

BY

S A RAHMAN,

AND

R N ABHYANKAR

(From the Department of Physiology, Osmania University Medical College, Hyderabad, Deccan)

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INTRODUCTION

OUR attention was first drawn to the fact that the frog's heart perfused with the ordinary Ringer's solution, generally mentioned in textbooks, did not continue to beat regularly for a very long time Sometimes the heart-beats were irregular from the very start and never became regular afterwards Hardikar's (1931) experience was also similar, so much so that he was forced to use defibrinated sheep's blood for his experiments

We started the present series of experiments in order to find out the comparative values of solutions of different formulæ, and to see which of them would give the best results In judging the efficacy of a solution the points considered were (*a*) the rates of the heart-beats, (*b*) the force of contraction, (*c*) the maximum duration in which the heart would go on contracting, and (*d*) if the solution in question could make the beats regular after they had become irregular by perfusing the heart with another solution

METHOD

The perfusions were made into the intact heart, the perfusion fluid entering the inferior vena cava and coming out through the aorta In some of the

* Read at the Section of Medical and Veterinary Research, Indian Science Congress, Bangalore, 1932

experiments the fluid was allowed to run out of the aorta, the heart being constantly perfused by means of fresh fluid from a large Mariotte bottle In other cases a closed circuit was established, the perfusion fluid being contained in a funnel and, after passing through the heart, being led back to the funnel through one of the branches of the aorta , the heart being thus perfused by the same fluid all the time The superior vena cava and the other branches of the aorta were, in all cases, ligatured to prevent the leakage of the fluid The difference in height between the level of the perfusion fluid and that of the sinus varied from half an inch to two inches in different experiments But in each experiment the levels were not allowed to vary, so that the perfusion fluid entered with a constant pressure all the time that the experiment lasted In those experiments also in which a closed circuit was not established and the fluid was allowed to run out of the aorta, arrangements were made to keep the perfusion-pressure constant

François Franck's cannula was used for perfusion the side tube of which was utilized to catch any air bubbles and to drain off the perfusion fluid while changing on to another Starling's heart lever was used so that the downstroke represented the contractions of the hearts in the graphs In all cases frogs were killed by pithing In each case the heart was prevented from drying from outside by pouring drops of the same solution over the apex as was used for perfusion

The following solutions were tried --

1 Ordinary Ringer, made up in the following manner To each litre of a 0 6 per cent solution of NaCl was added 10 c c of a 1 per cent solution of $NaHCO_3$, 10 c c of a 1 per cent solution of $CaCl_2$, 7 5 c c of a 1 per cent solution of KCl
2 Ordinary Ringer and blood, dilution 3 1
3 Bayliss Ringer consisting of NaCl 0 65 per cent, NaH_2PO_4—0 001 per cent, glucose—0 2 per cent, $NaHCO_3$—0 02 per cent, $CaCl_2$—0 012 per cent KCl—0 014 per cent
4 Bayliss' Ringer and blood, dilution 3 1
5 Bayliss' Ringer and urea in different concentrations
6 Bayliss' Ringer, modified by increasing the amount of $CaCl_2$ to 0 015 per cent, and the amount of KCl to 0 0175 per cent and by adding 0 001 per cent Na_2HPO_4
7 Bayliss' Ringer, but without glucose
8 Ordinary Ringer, with 0 2 per cent glucose

Altogether 25 frogs (*Rana tigrana*) were used Often the effects of more than one solution were tested on the same frog and the results compared All the experiments were carried out from July to November 1931

In the cases of frogs Nos 3, 13, 20, 21, 22, 23 and 24 the hearts were perfused with the ordinary Ringer In none of these cases did the hearts continue to beat regularly for more than one hour In the cases of frogs Nos 3, 20, 21 and 24, the

solutions were prepared in distilled water according to the directions given in text-books, and in the cases of the rest of the frogs, the solutions were prepared in tap-water Out of the four hearts perfused with the solutions prepared in distilled water in two cases, i e , Nos 3 and 21, the beats were irregular from the start and the rest began to beat irregularly in 20 minutes The rest of the frogs, in which the solutions used were prepared in tap-water, behaved in like manner The heart of frog No 13 began to beat irregularly in 45 minutes, that of No 22 was irregular in one hour and that of No 24 in 24 minutes Thus we noticed that the solutions made in tap-water were inclined to be less unsatisfactory than those made in distilled water

A qualitative analysis of the tap-water showed the presence of Na, K, Ca, chlorides, sulphates, carbonates, bicarbonates, iron and the presence of slight traces of nitrates, magnesium, aluminium and lead

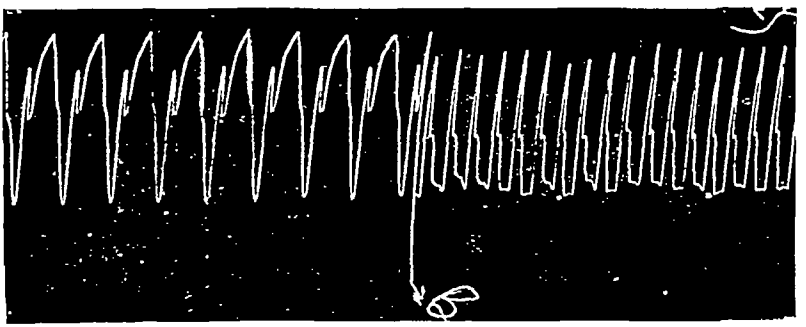

FIG 1 —The direction of the tracing is from left to right Up to the mark B, the heart shows an auriculo ventricular block of 2 1 At B, Bayliss' Ringer was substituted and the block at once disappeared (Downstroke represents systole) (Photograph—actual size)

In frogs Nos 20, 21, 23 and 24, after the heart-beats became irregular, Bayliss' Ringer was substituted for the ordinary Ringer, and in all these cases, as soon as the Bayliss' Ringer began to flow through the heart, the irregularity disappeared (Fig 1)

In the case of frog No 21, after the heart was made regular with Bayliss' Ringer, the ordinary Ringer was re-substituted and the heart went on regularly for about 3 hours more When again Bayliss' Ringer was substituted for ordinary Ringer to see if it would make any difference in the nature and rate of the contractions, we found that the heart rate increased from 31 to 42 beats per minute Another point elucidated by this experiment and confirmed by others to be described, was that once the heart was made to beat regularly for some time with Bayliss' Ringer, the substitution of ordinary Ringer did not affect the regularity of

the heart for a much longer period than was the case when ordinary Ringer was used from the very beginning

In the case of frog No 23, after the heart had been beating regularly with Bayliss Ringer for 50 minutes ordinary Ringer was substituted and the regularity was maintained for about 2½ hours when the heart became irregular again, Bayliss Ringer was re-substituted and the heart became regular at once, then again after 10 minutes ordinary Ringer was re-substituted and the regularity was maintained for half an hour more

Effect of blood

This was tried on four frogs Nos 2, 3, 8, and 9 respectively In all these cases sheep's defibrinated blood was used the blood was brought from the slaughter-house and was used 5 hours after it was shed In all cases the blood was diluted with 3 times its volume of Ringer's solution In the cases of frogs Nos 2 and 3, the hearts were perfused first with ordinary Ringer made with distilled water in both the cases the beats were irregular from the start, then blood diluted with ordinary Ringer was substituted, the beats at once became regular, the contractions became stronger and the rates rapid In the case of frog No 3, ordinary Ringer was re-substituted, the heart rate became slower and the contractions feeble, after 15 minutes Blood Ringer was again substituted and the heart-beats showed the same increased effects

In cases of frogs Nos 8 and 9, in which Bayliss' Ringer made with tap-water was used instead of the ordinary Ringer for diluting the blood same effects were noticed

Fig 2 shows the effect of substituting Blood Ringer The rate increased from 45 to 70 per minute and the range also In the case of frog No 8, the same experiment was repeated with the same results In the case of frog No 9 continued perfusion with Blood Ringer made the heart-beats gradually slower both as regards rate and range, and the substitution of fresh Blood Ringer made no difference to it, finally the heart-beats became irregular in about 2 hours, then we substituted Bayliss' Ringer alone and the heart became at once regular, after this we substituted undiluted sheep's defibrinated blood and within 5 minutes the heart stopped in systole In the case of frog No 9, we made sure that the blood was not hæmolysed throughout the experiment This was important as we know from the works of Gothlin (1902), (Luciani, 1911), that hæmolysed blood is injurious to the heart Two inferences could be drawn from these experiments (1) Blood Ringer does increase the rate and range of the heart's contractions, and (2) that it is inferior to Bayliss' Ringer so far as the survival period of the heart is concerned the second inference being based only on one experiment, cannot be regarded as conclusive

We are not in a position to explain why the addition of blood to Ringer brings about increase in rate and range of contractions Reference to the old literature shows that many experiments were conducted to elucidate this point, but most of them were unsuccessful Baglioni (Luciani, 1911) working on the isolated heart of Selachian, found that it was possible to keep it alive for a prolonged period by treating it with a solution containing 2 per cent urea and 2 per cent NaCl He concluded from his experiments, that urea promotes the contraction of the heart and that a larger percentage arrests the heart in systole (Baglioni, 1907) Lambert (1905) (Luciani, 1911) confirmed the favourable action of urea on frog's heart, Baglioni and Federico (1906) (Luciani, 1911) on that of the toad In these animals they found that urea increases the intensity and duration of the systolic phase

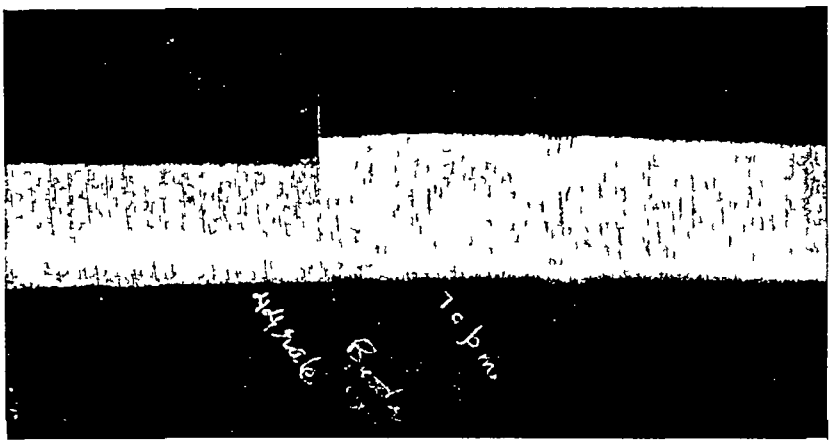

FIG 2 —The direction of the tracing is from left to right The tracing shows the effect of the substitution of the solution of blood on the contraction of the heart, the rate has increased from 44 to 70 beats per minute and the excursions of the lever have also increased (Photograph— actual size)

We tried the effect of urea on frogs Nos 11, 12, 24 and 25 and the results obtained were variable, in some cases the range increased while in others just the opposite effect was noticed In the last two cases, the experiment was done repeatedly, but either there was no effect at all or the results were so variable that it could not be concluded whether urea had any definite action, either on the rate or on the nature of contractions

PERFUSION WITH BAYLISS' RINGER

In cases of frogs Nos 5, 6 and 7, the perfusion was made all along with Bayliss' Ringer in order to see how long the heart could be made to contract regularly

In all these cases the open method was used, i e , the perfusion fluid was allowed to escape through the aorta as was done in experiments with ordinary Ringer The perfusion fluid was kept in a big Mariotte bottle and the perfusion-pressure was arranged to remain constant In all these cases, the heart was kept moist from outside by dropping Bayliss Ringer on the apex

In frog No 5, the experiment was started at 11-15 A M and closed at 3-15 P M , heart still regular

In the case of frog No 6, the experiment was started at 11-15 A M , the heart remained irregular till 2 P M owing to the presence of a blood-clot in the cavity of the heart , at 2 P M this clot was expelled through the aorta, the heart-beats then became quite regular and remained so till 6-10 P M when the experiment was stopped , the pH of this solution was 7 25

In the case of frog No 7 the experiment was started at 9-30 A M and stopped at 10-15 P M , the heart was quite regular throughout The pH of the solution in this case was tested by Dr Qureshi about 5 days after the experiment and was found to be 7 05

Perfusion with Bayliss' Ringer modified by increasing the amount of Ca and K and by adding Na_2HPO_4

As the pH of the Bayliss' Ringer which we prepared was low (the actual pH mentioned by Bayliss is 8 4) we tried to see the effect of a more alkaline solution by adding Na_2HPO_4, at the same time we tried to see the effects of increase in the quantities of Ca and K The new solution thus obtained had the following composition —

$NaCl = 0\ 65$ per cent, $NaHCO_3 = 0\ 02$ per cent, $CaCl_2 = 0\ 015$ per cent, $KCl = 0\ 0175$ per cent, $NaH_2PO_4 = 0\ 001$ per cent, $Na_2HPO_4 = 0\ 001$ per cent, Glucose $= 0\ 2$ per cent

This perfusion fluid was used in the cases of frogs Nos 10 and 12 and we saw that as compared to Bayliss' Ringer, perfusion with this fluid made no marked difference as regards the rate and range of the contractions

Effect of glucose

As the main difference between the formula given by Bayliss and the other formulæ which we found to be inferior, was the presence of glucose, we conducted a few experiments to test the effect of glucose

In cases of frogs Nos 14, 15, 16, 17, 18, 19 and 22, the effect of glucose was tested In all these cases, the closed circuit system was used Oxygen was passed through the solutions which were made in tap-water

In the case of frog No 14, the perfusion with Bayliss' Ringer without glucose kept the heart regular for about 4 hours, then it became irregular The substitution of Bayliss' Ringer with glucose made the heart-beats regular at once.

In the case of frog No 16, Bayliss' Ringer without glucose was used, the heart-beats became irregular in about 3 hours

In the case of frog No 17, perfusion was started with Bayliss' Ringer without glucose, and the heart became irregular within 15 minutes, the substitution of Bayliss' Ringer with glucose made the beats regular at once The same experiment was repeated in this frog the heart being perfused repeatedly with the solution without glucose and when the beats became irregular, substitution of the solution with glucose made them regular at once

In the cases of frogs Nos 18 and 19, similar experiments were performed, the heart being perfused with the solution without glucose, the heart-beats became irregular in 25 minutes Then the same solution was substituted, with the idea of eliminating the possibility of the mechanical action, concerned with the changing

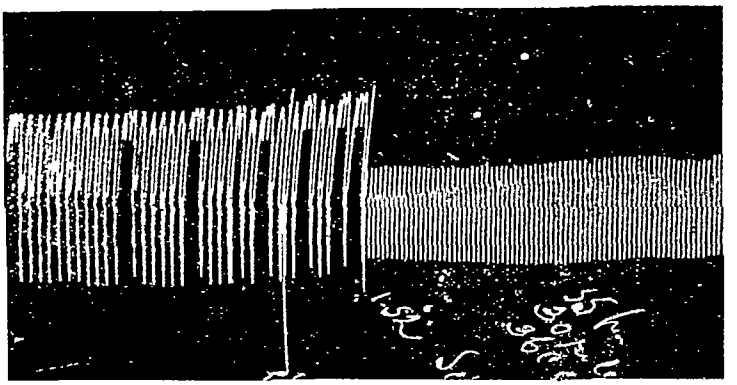

FIG 3 —The left side of the tracing shows the irregular contractions of the heart after perfusing it for some time with the ordinary Ringer Substitution of the same solution but containing 0 2 per cent glucose makes the heart beats regular again (Photograph—actual size)

of solutions, being a factor in bringing about the regularity of the heart-beats We found that this made no difference and the heart-beats remained irregular Then we substituted Bayliss' Ringer with glucose and the beats became regular at once

In the case of frog No 22, the experiment was started with perfusing the heart with ordinary Ringer The heart-beats became irregular in 45 minutes, then the same Ringer containing 0 2 per cent glucose was substituted and the beats became regular at once (Fig 3)

Thus, in all these cases we found that glucose had a definite effect in not only making the heart beat regularly for a longer time, but also that when the beats had become irregular, by perfusing it with a solution not containing glucose, the

substitution of the same solution with glucose brought about the regularity This we noticed constantly

This is interesting in view of the recent work by Clark Gaddie and Stuart (1931) who working on *Rana esculanta* (Hungarian) have found that hearts perfused with Ringer's fluid without glucose survive for a longer period than those perfused with Ringer containing glucose This is in keeping with their findings that for the first six hours of perfusion the frog's heart hardly uses up any glucose at all

SUMMARY AND CONCLUSIONS

Altogether 20 frogs were utilized Solutions of different formulæ were used and results on the rate and range of the heart-beats of *Rana tigrina* were compared

(*i*) Sheep's defibrinated and diluted blood was found to increase both the rate and range of heart-beats in a manner which was not obtained with other solutions

(*ii*) Bayliss' solution was found to give the most satisfactory results

(*iii*) Glucose was found to be of definite advantage, not only in prolonging the survival period of the heart but also in bringing about the regularity of the beats after they had become irregular by using a solution not containing glucose

(*iv*) Solutions made in tap-water were found to give better results than those made in distilled water

(*v*) Urea was not found to have any definite effect

We wish to express our thanks to Dr S W Hardikar for the helpful criticisms, to Dr Qureshi for estimating the pH values of the solutions, and to Mr Ghouse Mohiuddin for making the qualitative analysis of the tap-water

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THE INFLUENCE OF H ION CONCENTRATION ON CHOLERA BACTERIOPHAGY

A PRELIMINARY NOTE

BY

R SANJIVA RAO, MB, BS, DTM

(*From the King Institute, Guindy*)

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INTRODUCTION

ASHESHOV (1930) has classified cholera bacteriophage into three distinct types based on 'group characteristics which are not limited to the reciprocal action on the secondary cultures only'

Type A is the quick-acting type attacking only the smooth and not the rough strains The group however is not homogeneous, for the different members vary in their range of activity and stability

Types B and C are slower in action but more stable (particularly B) They are omnivirulent, i e, they have a very wide range of activity, attacking both rough and smooth strains

The restricted range of activity of type A led Asheshov to adopt his original type A of cholera bacteriophage (61 A) to lyse the resistant smooth vibrio, and the resulting 'phage was numbered 62 A This process of adoption continued as he met more resistant smooth cholera vibrios and, in his report for 1929-30, he states that his observations suggest the existence of four groups of cholera vibrios which may be represented as follows —

	Group I	Group II	Group III	Group IV
Cholera bacteriophage 61 A	+			
do 62 A	+	+		
do 63 A	+	+	+	
do 64 A	+	+	+	+

Subsequently in his report for 1930-1931, he mentions that he has found six vibrios of smooth character which were resistant to cholera bacteriophage 64 A

While it is quite likely that more and more groups may be added on to the present number it is highly probable that differences in environmental conditions can be held to account for the different groups of vibrios that we come across

To ascertain if the H ion concentration has any influence on the grouping of the vibrios and the phenomenon of cholera bacteriophagy in general, the following experimental work was carried out —

Material and methods employed

1 One strain of each of the four groups of cholera vibrios namely —

Ch J (group I) Ch 782 (group II) Ch Madras I (group III) and Ch Puri 5 (group IV) obtained from Dr Asheshov

2 Ch strains '56' and '68 isolated recently in Ramnad and Madras respectively

3 Cholera bacteriophages 61 A, 62 A, 63 A and 64 A corresponding to the four groups of vibrios and 146 B and 13 C also obtained from Dr Asheshov All the cholera strains were maintained in the laboratory on papain agar (pH 6 8) and the 'phages were cultivated in papain broth (pH 8 4) on the cholera strain J The platings were done on peptamin agar medium

All the above-mentioned media are those employed by Dr Asheshov in his cholera bacteriophage work

For the present work two sets of papain agar media, one set of pH 6 6 and the other of pH 8 4 were prepared Two sets of one per cent Difco peptone water (pH 6 6 and pH 8 4) were also prepared for emulsifying the acid and alkaline cultures respectively

For plating the technique was the same as the one employed by Dr Asheshov for his cross-tests The peptamin agar plate was previously marked on the outside by a grease pencil into squares The cultures were then streaked vertically by a special loop designed by Dr Asheshov and the plate was allowed to dry in the incubator for 10 to 15 minutes After drying a drop each of the 61 A to 64 A type of cholera bacteriophage was put in each square in four horizontal rows, so that each streak of culture was tested against all the four 'phages 61 A to 64 A The plates were then incubated at 37°C and the results read the next morning

Experiments with acid media.

The four cholera strains J, 782, Madras I and Puri 5, representing the four groups of cholera vibrios, were inoculated on to papain agar pH 6 6, with bromothymol blue as indicator As marked alkalinity during the first few days is one of the features of the metabolic process of *V cholera*, the inoculation on the agar slope

was confined to one small round area so that the growth of *V cholera* might not considerably diminish the acidity of the medium For the same reason the cultures were aged for about 6 weeks, when the preliminary alkalinity had disappeared and given way to acidity Thus after 6 weeks on acid agar the cultures were emulsified in Difco peptone solution of pH 6 6 and incubated at 37°C They were then plated and tested against cholera bacteriophage 61 A to 64 A as stated above after 5 hours, 24 hours, 48 hours and 96 hours, with the following results —

PLATE I

5 hours after inoculation in peptone

	J	782	M 1	P 5
61 A	O	O	O	O
62 A	O	O	O	O
63 A	O	O	O	O
64 A	O	O	O	O

PLATE II

24 hours after inoculation in peptone

	J	782	M 1	P 5
61 A	O	O	O	O
62 A	O	O	O	O
63 A	O	O	O	O
64 A	O	O	O	O

PLATE III

48 hours after inoculation in peptone

	J	782	M 1	P 5
61 A	O		—	—
62 A	O	O	O	—
63 A	O	O	O	O
64 A	O	O	O	O

O indicates area of complete lysis

PLATE IV

72 hours after inoculation in peptone

	J	782	M 1	P 5
61 A	O	—	—	—
62 A	O	O		—
63 A	O	O	O	—
64 A	O	O	O	O

indicates area of incomplete lysis

PLATE V

96 hours after inoculation in peptone

	J	782	M. 1	P 5
61 A	O	—	—	—
62 A	O		—	—
63 A	O	O	O	—
64 A	O	O	O	(

The pH of the suspension was tested after 72 hours and was found to have altered to 7 4 from 6 6

Experiments with alkaline media

The cultures which were aged on acid agar were transferred to alkaline agar pH 8 4 The whole area of the slope was inoculated so that alkalinity might be enhanced as much as possible during the growth of the vibrios Such cultures were done daily for a period of 12 days and then the cultures were emulsified in Difco peptone solution of pH 8 4, incubated at 37°C and then plated against cholera bacteriophage 61 A to 64 A, after 5 hours, 24 hours, 48 hours, 72 hours and 96 hours, with the following results —

PLATE VI

5 hours after inoculation (pH 8 4)

	J	782	M. 1	P. 5
61 A	O	O	O	—
62 A	O	O	O	O
63 A	O	O	O	O
64 A	O	O	O	O

PLATE VII

24 hours after inoculation

	J	782	M 1	P 5
61 A	O	—	—	—
62 A	O	O	O	—
63 A	O	O	O	—
64 A	O	O	O	O

PLATE VIII

48 *hours after inoculation (pH 8 4)*

	J	782	M 1	P. 5
61 A	O	—	—	—
62 A	O	O	—	—
63 A	O	O	O	—
64 A	O	O	O	O

PLATE IX

72 *hours after inoculation*

	J	782	M 1	P 5
61 A	O	—	—	—
62 A	O		—	—
63 A	O	O	O	—
64 A	O	O	O	O

PLATE X

96 *hours after inoculation*

	J	782	M 1	P 5
61 A		—	—	—
62 A	O	—	—	—
63 A	O	—		—
64 A	O	—		—

Experiments with cholera bacteriophage type B resistant strains

Asheshov states that cholera bacteriophage B is omnivirulent and that he has not come across a ' single typical vibrio which was not attacked by one and the same 'phage of this type ' However, during a recent small epidemic of cholera in this Presidency, several strains were collected some of which were lysable by cholera bacteriophage 64 A and bacteriophage 13 C but not by 146 B They were all typical vibrios, motile, smooth (by Millon's test), highly agglutinable by the standard Lister Anti-serum and fermented glucose, maltose saccharose and mannite without gas Two of these strains Nos ' 56 ' and ' 68 ' were then chosen for experiments They were tested and found to be free from any contaminating bacteriophage The test also revealed the interesting fact that cholera bacteriophage 146 B could lyse the secondaries arising after the action of cholera

bacteriophage 64 A, but not after the action of cholera bacteriophage 13 C on the two strains. The following plates illustrate the point —

PLATE XI

Action of cholera 'phages A B and C on Ch '68

	A	B	C
Ch. '68'	O	—	O

PLATE XII

Secondaries after the action of ch 'phage on ch '68'

	XA	XB	XC
64 A	—	O	O
146 B	O	—	—
13 C	O	O	—

XA Secondary of '68' after the action of ch 'phage 64 A, etc

These two strains '56' and '68' were cultivated in papain agar pH 8·4 and sub-cultures were made daily for a period of 10 days at the end of which they were emulsified in Difco peptone pH 8·4. The suspensions were incubated and then plated and tested against cholera bacteriophage 64 A, 146 B and 13 C, at the end of 24 hours, 48 hours, 72 hours and 96 hours, with the following results —

PLATE XIII

24 hours

	A	B	C
'56'	O	—	O
'68'	O		O

PLATE XIV

48 hours

	A	B	C
'56'	O		O
'68'	O		O

PLATE XV

72 hours

	A	B	C
'56'	O		O
'68'		O	O

PLATE XVI.

96 hours

	A	B	C
'56'		O	O
'68'	—	O	O

Comments

It is obvious from the experiments conducted on acid and alkaline media that the phenomenon of cholera bacteriophagy is intimately connected with the pH of the medium in which the organism is cultivated

Plates I and II show that on continued existence in an acid environment the groups II, III and IV have all become group I, in other words have all become lysable by cholera bacteriophage 61 A In Plate III, however, a tendency is shown to revert to their original groups In Plate IV, they have practically fully reverted to their original groups and, in Plate V, group II is only incompletely lysed by 62 A and 63 A, while group IV is incompletely lysed by 64 A This is easily explained by the fact that growth of cholera vibrio is associated with increased alkalinity The pH of the Difco peptone before inoculation was 6 6 and 72 hours after inoculation was 7 4 Evidently the influence of altered H ion concentration is exerted more vigorously in a fluid than in a solid medium, and the short time in which the vibrios have been in the alkaline peptone has undone the change wrought by prolonged growth on a solid acid medium

Plate VI shows the influence of daily sub-culture on alkaline agar (pH 8 4) for a period of 12 days The immediate change (5 hours) is very little except in the case of Ch Puri 5, which now has come down to group II Plate VII shows the additional influence of alkaline peptone, the original alkalinity of which has been enhanced by the growth *V cholera* Plate VIII shows all the vibrios in their original groups In Plate IX, group II vibrio has shown a tendency to become group III vibrio while, in Plate X, group II and group IV vibrios are not at all lysable by cholera bacteriophage 64 A, group III vibrio is only faintly lysable by 64 A and group I vibrio shows a tendency to become group II vibrio

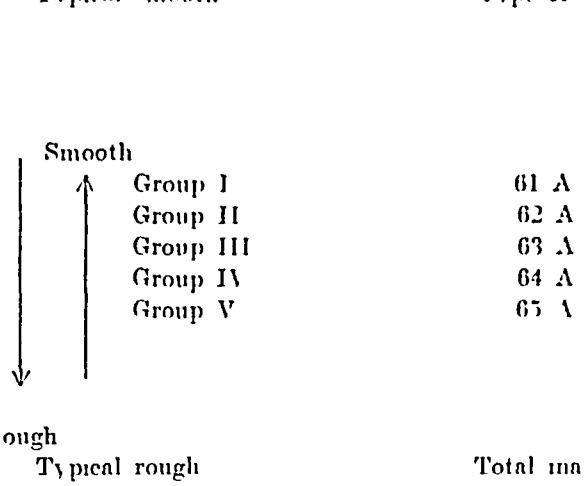
As has been observed above, the altered pH has been speedier in its effects in liquid than in solid medium

Asheshov's experiments in connection with the preservation of cholera vibrio as near to their natural state as possible have led him to the conclusion that progress towards the rough phase occurs quickly in alkaline medium

The experiments detailed above have shown that in alkaline medium cholera vibrios tend to arrange themselves in different groups according to their lysability by one or more 'sub-types' (61 A, etc) of the A type of cholera bacteriophage It is then logical to presume that the different groups of cholera vibrios are only different grades of roughness, the differences in roughness among the groups close to each other being too inappreciable to be detected by Millon's test

Type A cholera bacteriophage is not known to lyse rough cholera vibrios There is, however no sharp line of demarcation between the typical smooth and the typical rough So theoretically there can be an infinite number of different grades between the two, depending upon the proportion of smooth to rough

elements in the individual vibrio Then the different groups mentioned by Asheshov are probably synonymous with these different grades of roughness This idea can be represented as follows —



That even in nature the type A 'phage tries to adopt itself to resistant vibrios is shown by the isolation of cholera bacteriophage 64 A in nature in Madras (Pandit and Rao)

Plates XIII to XVI demonstrate the influence of alkalinity on lysis by cholera bacteriophage B Ch '56' has in 96 hours acquired lysability by 'phage B, while ch '68' has acquired the same change in 72 hours At the same time lysability by cholera bacteriophage 64 A has suffered deterioration This only confirms the results obtained from the previous experiments

Summary

1 The relationship between cholera bacteriophagy and the H ion concentration of the medium in which the cholera vibrio is cultivated has been studied

2 Within the optimum limits lysis by the type A of cholera bacteriophage is enhanced by increasing acidity and that by the type B by increased alkalinity

3 As a tentative conclusion it appears probable that the groups into which Asheshov classed the cholera vibrios are only different grades of roughness and can be altered appreciably by changing the pH of the medium in which the vibrios are grown, but further work is required to establish this important conclusion

Acknowledgment

My thanks are due to Lieut-Colonel H H King, I M S, Director, King Institute, Guindy, for kind encouragement and advice throughout the work, to

Dr Asheshov of the Bacteriophage Inquiry for the materials kindly supplied by him, and to Dr C G Pandit, Assistant Director, King Institute, for the very valuable training he gave me in bacteriophage work

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Ind Jour Med Res, XX, 2, *October*, 1932

OSTEOMALACIA (LATE RICKETS) STUDIES

Part X

ADMINISTRATION OF CALCIUM PHOSPHATE

BY

DAGMAR CURJEL WILSON, M D (Glas), D P H (Camb), (*Retd*),
In-Charge, Osteomalacia Inquiry, Indian Research Fund Association

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Introduction

In northern India rickets in its various forms, osteomalacia, late rickets and infantile rickets, tends to occur both in rural and urban areas wherever there is extreme deficiency of diet or sunlight, or more frequently where there is a relative deficiency in both these factors Improvement in diet only leads to improvement in symptoms when sunlight is available, and conversely, the provision of sunlight for cases on deficient diet is of little avail (Wilson, 1931*a*) An analysis of 400 urban cases of late rickets and osteomalacia shows that about half the patients live under social conditions, which do not admit of an altered mode of life and tend to relapse after treatment owing to want of sunlight and of a balanced diet, while in rural areas field workers suffering from late rickets and osteomalacia, though obtaining adequate sunlight, are not in an economic position to improve their dietary deficiencies (Wilson, 1931*c*) All such cases need vitamin D as calcifying substance and in its absence utilization of calcium and phosphorus is impaired, in view of the very small amount of vitamin D often available it is necessary to ensure adequate intake of these minerals Examination of the blood chemistry of cases of osteomalacia and late rickets in India have shown a low serum inorganic phosphorus (Wilson, 1931*b*, Hughes *et al*, 1929, 1930 and 1931) which may be associated with a low serum calcium and with tetany

I am indebted to workers at the Lister Institute for advice regarding a suitable salt, and to the Medical Research Council for the provision of sufficient amounts of drugs for the purposes of the experiment

Methods.—The neutral precipitated tricalcium* phosphate was used for the therapeutic experiment and was administered daily in doses of grains 45 to 60, in a glass of water, solution being aided by the addition of dilute hydrochloric acid minims 30. (In a few instances owing to digestive disturbance, the acid was omitted from the solution with no apparent adverse effect.)

Forty-one cases of late rickets and osteomalacia (ages 9 to 45 years, females 40, male 1), were kept under observation in Lahore City and also in the Palampur *tehsil* of the Kangra valley, an area where as previously detailed many field workers are living under abnormal dietary conditions with deficiency of vitamins† A, D and salts (Wilson, 1931).

Before treatment the clinical condition of each patient was classified according to the degree of severity +, ++, +++, these headings being comparable to those used in previous records and depending on the incidence, situation and amount of pain and deformity and whether tetany were present, the length of treatment was also based on previous experience (Wilson, 1931*a*). Dietary and living conditions were noted in each case and the patients divided into two groups (1 and 2), according to whether the deficiency was in diet alone or in both diet and sunlight.

After three weeks' treatment it was found that the clinical condition of five severe cases who lived a crippled life indoors, became worse and their treatment with the salt was discontinued. (They subsequently improved when given irradiated substances containing vitamin D.) There remained 36 patients in the two groups, each group was subdivided in two classes (*a* and *b*) according to whether the patients were able to move about or were crippled. At the end of the six weeks' observation period each patient was re-examined and any alteration in the clinical condition noted.

Results.—The effect of treatment with calcium phosphate is shown in the following table.

Ten control cases in (*a*) urban and (*b*) rural areas who received either radiostoleum ½ c.c., or cod-liver oil 2 drachms daily, showed satisfactory improvement within the experimental period of six weeks.

Discussion

The results of group I (a).—Fourteen cases on deficient diet who lived actively out of doors, showed at the end of six weeks' treatment nine patients entirely free of symptoms (three of whom were in the latter months of pregnancy, a time when symptoms usually become worse), and five who were much improved.

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* Supplied by the British Drug Houses, Limited.

† The deranged defence mechanism to infection due to perverted metabolism in this area is also shown by a survey of school girls, over one half of those examined showing signs of malnutrition and of rickets. Leprosy also, a disease in which deficiency of diet is known to be a factor, has a high incidence in this district.

TABLE

Administration of neutral precipitated tricalcium phosphate

	Group I Deficient diet												Group II Deficient diet + deficient sunlight											
	(*a*) Patient active						(*b*) Patient crippled						(*a*) Patient active						(*b*) Patient crippled					
	+++	++	+	*Nil*	Tetany present	Total cases	+++	++	+	*Nil*	Tetany present	Total cases	+++	++	+	*Nil*	Tetany present	Total cases	+++	++	+	*Nil*	Tetany present	Total cases
Before treatment, degree of severity of clinical condition	3	10	1		2	14	2	11			2	3	7	6	3		2	16	2	1			1	3*
After six weeks' treatment, degree of severity of clinical condition			5	9†		14	2		1		1	3	6	3	6	1		16	2	1			1	3

* Plus 5 cases who ceased treatment on getting worse after 3 weeks

† Include 3 cases in latter months of pregnancy

While in group I (b)—three crippled women only able to sit out of doors in the sunlight showed little change in their clinical condition, in one case tetany persisting at the end of six weeks' treatment

In group II (a)—Sixteen cases, deficient both in diet and in sunlight but able to move about in their homes, showed slight improvement at the end of the experimental period *But in group II (b)*—the condition of three crippled patients deficient in diet and sunlight was unaffected by treatment, and in one case tetany continued throughout the six weeks showing that calcium was not being absorbed, or not made available for use in the body

Conclusions

(1) In field workers living on a diet relatively deficient in vitamins A, D and salts, the addition of calcium phosphate in doses of drachm 1 daily over a period of six weeks will cure or much improve the pain and deformity of late rickets and osteomalacia It has not yet been determined whether calcium or phosphorus is the preponderating missing factor in such cases

(2) This treatment is unsuitable for cases whose crippled condition does not permit of muscular and circulatory activity or who in addition to a deficient diet also suffer from want of sunlight, in such cases the addition of vitamin D is also necessary for cure

Climatic conditions throughout the experiment were noted daily, and remained equal during the period under observation Seasonal variations of temperature did not, therefore, in any way affect the results obtained

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Idem (1931*b*)	*Ibid*,		p 969
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Ind Jour Med Res, XX, 2, October, 1932

STUDIES IN PERNICIOUS ANÆMIA OF PREGNANCY

Part V

PRODUCTION OF A MACROCYTIC ANÆMIA IN MONKEYS BY DEFICIENT FEEDING

BY

LUCY WILLS, M A (Cantab), M B, B S (Lond),

AND

H S BILIMORIA, M B, B S, D T M,

Maternal Mortality Inquiry, Indian Research Fund Association

(*From the Haffkine Institute, Bombay*)

[Received for publication, April 29, 1932]

In a previous series of experiments one of us (Wills, 1930*a*) showed that it was possible to produce a severe macrocytic anæmia in rats by deficient feeding, but this anæmia, though it only occurred in the ill-fed animals, was associated with an infection with *Bartonella muris ratti*. As the diet fed in these experiments was based on one in common use among sufferers from pernicious anæmia of pregnancy, the possibility of a similar infection in such cases was considered, and an exhaustive, but fruitless search made for any evidence of such an infection. The possibility of the infection in the rat being secondary to the anæmia and not, as originally thought the cause, was then considered and the present series of experiments planned to see if a similar dietetic anæmia could be produced in monkeys. Monkeys were chosen as they do not suffer from Bartonella infections, even after splenectomy.

Experimental

The 12 experimental monkeys (*Macacus sinicus*), with one exception, an old male (No. 0) who had been in the Institute for several years, were immature animals, newly caught and quite wild at first. They were kept in separate cages in a large airy room and were set free for exercise for a couple of hours every other day, and on the alternate days were exposed to the early morning sun for the same time

The animals under these conditions remained active and free from rickets, and, with a few exceptions from intercurrent infections The experiment ran for 15 months so that some of the animals were subjected to all the climatic variations of the year

The 12 animals, numbered 0—9 and 1*a* and 2*a*, were all fed on a basal diet consisting of polished rice 30 parts, white bread 32 5 parts, wheat *chapatti* 12 5 parts, *ghi* 10 parts, white pumpkin 10 parts and meat 5 parts This diet is based on one in common use among Mohammedan women patients in Bombay The ingredients were bought in the bazaar and the colour tests for vitamins A and D were negative in all the samples of *ghi* tested The rice, meat and pumpkin were cooked together with a little common salt and then intimately mixed with the remaining constituents of the diet The diet was fed in excess, and unlimited tap-water supplied to the animals In addition the animals were fed on certain extra rations as shown in Table I The milk received by numbers 8 and 9 was not given by pipette but in a dish, and was not always completely finished, though the animals generally took it readily

TABLE I

Number of monkey	Daily additions to basal diet					Anæmia	Survival time in days
	Orange, g	Tomato or carrot, g	Cod liver oil, c c	Milk, c c	Yeast powder, g		
0	10					++	162
1	10				1	+	173
1*a*	10				1	−	66*
2	10					++	176
2*a*	10					+	114
3	10				1	++	169
4	10					+	174
5		20	2			+	173
6		20	2			++	200
7		20	2		1	++	436†
8		40	2	60	1	−	436†
9		40	2	60		++	436†

* Died from intercurrent diarrhœa † Experiment ended

The blood for examination was taken from the ear vein The methods were the same as those used in the previous work (Wills and Mehta, 1930*a*), except that the hæmoglobin was estimated as acid hæmatin in a Leitz colorimeter against a known acid hæmatin solution, standardized by the oxygen combining capacity method of Van Slyke

Through the kindness of Mr Laud, Superintendent of the Victoria Gardens, Bombay, a series of normal monkeys was studied and standards established (Bilimoria, 1931)

Results

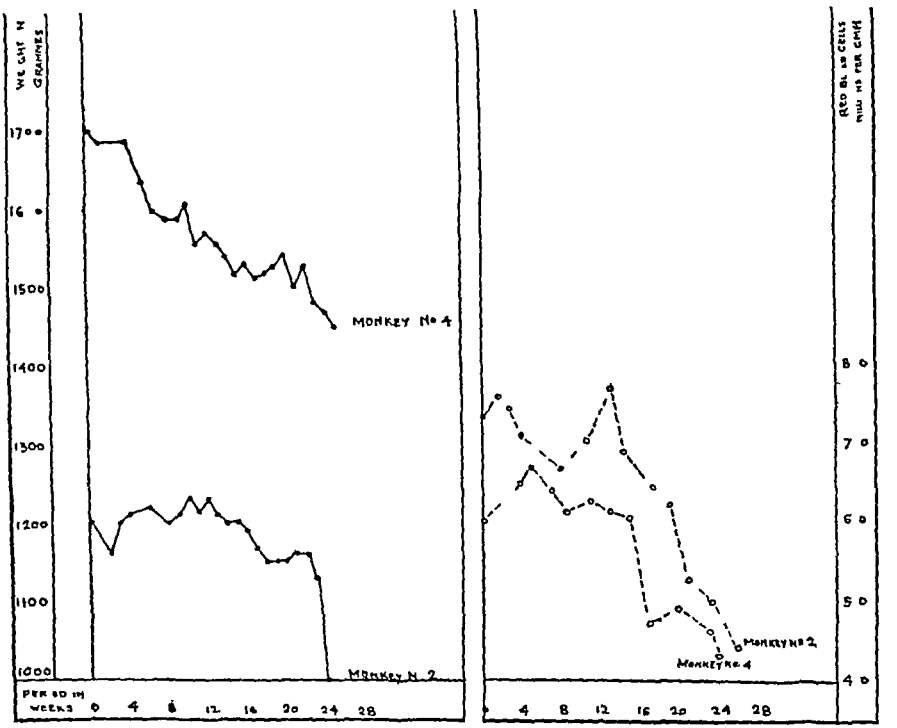
The animals were divided into four groups, according to their diet, and these groups will be considered separately

Group 1.—Basal diet + 10 g orange

Four animals, Nos 0, 2, 2*a* and 4, received this diet All died within 176 days of starting the diet, all failed to grow, and all developed some degree of anæmia (Chart 1) Post-mortem there were no lesions to account for death, the only

Chart 1

Weights and Red Blood Cell counts of monkeys Nos 2 and 4



abnormal findings being the extreme degree of wasting, a mild degree of visceral siderosis, and remarkable hyperplasia of the marrow of all the bones The type of anæmia present and the marrow change will be considered in greater detail later

Group 2.—Basal diet + 20 g of tomato or carrot + 2 c c cod-liver oil

Two animals, Nos 5 and 6, formed this group They behaved in every respect like those in group 1 except that one animal survived for 200 days, showing that the dietary deficiency was not one of vitamins A and C

Group 3.—Basal diet + 10 c.c. orange juice + 1 g. Harris's concentrated yeast powder.

Nos. 1, 1*a* and 3 formed this group. These animals, with the exception of No. 1*a* which died of an intercurrent attack of diarrhœa, behaved like, and grew

CHART 2

Weights and Red Blood Cell counts of monkey No. 7

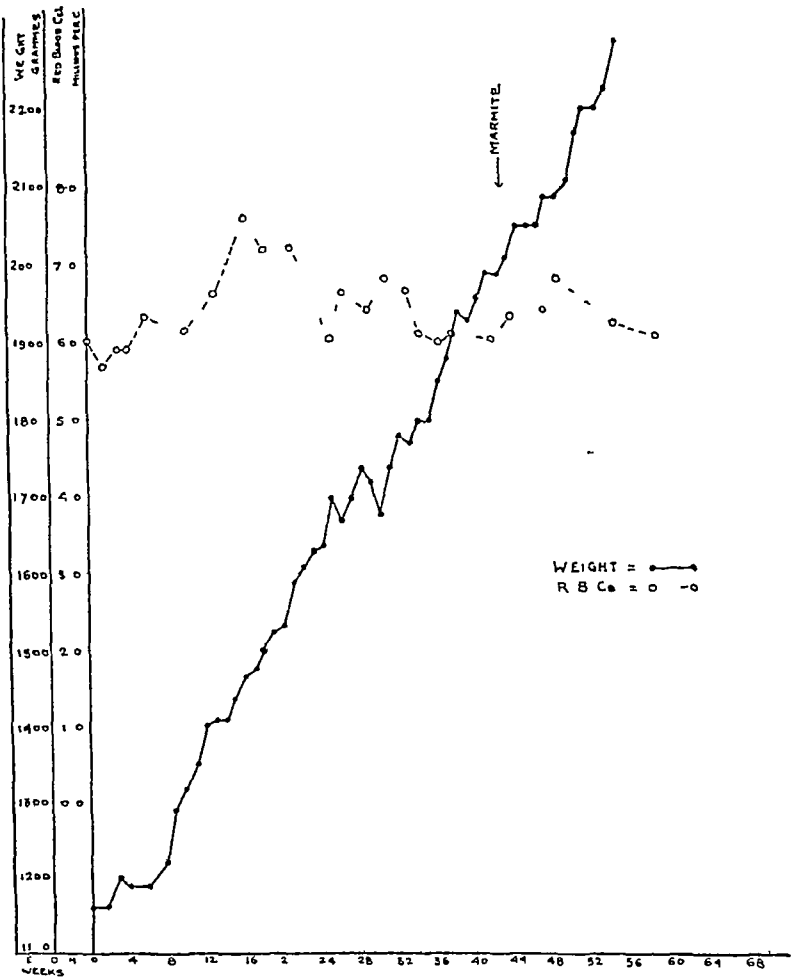
no better than, the animals in group 1 on the same diet without the addition of yeast, so that either the addition was too small to be beneficial, or the vitamin B complex was not concerned

Group 4.—Basal diet + 2 c c cod-liver oil + other rations as detailed below

This group is made up of the three animals, Nos 7, 8 and 9, which survived the experiment, one as the result of treatment and the other two on their original diet

CHART 3

Weights and Red Blood Cell counts of monkey No 8



(Table I) Each animal will be considered separately No 7 which received the Harris's yeast powder as an extra ration, but no milk and only the smaller ration of fruit, grew very poorly (Chart 2), and his red cell count fell from 7 1 million to 3 5 million per c mm When the yeast powder was changed to a larger dose of Marmite

(a yeast extract), this monkey not only started to grow immediately but his anæmia cured rapidly

No 8 which received milk a double fruit ration and yeast powder, not only grew well but remained in perfect health throughout the experimental period

CHART 4

Weights and Red Blood Cell counts of monkey No 9

No 9, which received milk and a double fruit ration but no yeast, grew well for the first 30 weeks, but became anæmic during this period On the 224th day of

the diet, he developed an acute attack of diarrhœa and lost weight rapidly, this attack was treated by liver extract (B D H fluid extract 2 c c a day) for six days, when the diarrhœa stopped and the weight immediately began to rise again In spite of this treatment the blood picture deteriorated steadily for the next 38 days (Chart 4), when for no discoverable reason, the blood count began to rise till it reached normal limits 2 months later

These three monkeys, unlike all the other experimental animals, survived for a considerable period, but, as shown above, their condition varied No 7 did not grow, and became anæmic, but was cured by Marmite, No 8 grew and remained in perfect health and No 9 grew but became anæmic, and then recovered spontaneously These findings suggested that there was some common factor present in yeast and milk, possibly the vitamin B complex or some factor associated with this complex, that, given in large enough doses, protected the animals from anæmia The spontaneous cure which occurred in No 9 might be accounted for by an improvement in the vitamin B content of the milk, or a better taking of that ration, which, not being forcibly fed, was taken in varying amounts Another possibility was that the monkey, which was known to eat his own fæces, was cured by this means (Roscoe, 1931) Whatever the explanation of the cure, the findings in these three animals, especially the dramatic cure of No 7 by Marmite, were sufficiently suggestive to lead to a trial of this yeast extract in cases of tropical macrocytic anæmia, where it proved a most efficacious remedy (Wills, 1931)

Blood and bone-marrow changes

The anæmia, which occurred in all the animals except No 8, was of the same type but the most marked changes were seen in Nos 7 and 9, which survived longer than the other animals Table II shows the changes that occurred in these two monkeys during the experimental period and, in addition, normal values, the average of the findings from 27 healthy animals (Bilimoria, 1931) Chart 5 gives the Price-Jones curves of No 7 taken before and at the height of the anæmia

The anæmia was a macrocytic one There was marked anisocytosis, the Price-Jones curve showing a shift to the right as the anæmia developed and a very definite increase in the variability There was little poikilocytosis but some polychromatophilia and stippling of the red cells There was no hæmoglobin lack, the colour index was never depressed and increased as large cells appeared in the blood Megaloblasts were found at the height of the anæmia but normoblasts were more frequent, the anæmia in this respect resembling tropical macrocytic anæmia rather than true pernicious anæmia (Wills, 1930) The variability in the white cell count of normal monkeys is so great that it is very difficult to draw any conclusions without a large number of animals but in all the monkeys the count was decreased at the height of the anæmia

TABLE II

Blood changes in anæmic monkeys

	Date	Red blood cells per c mm *	Hæmoglobin per cent *	Colour index *	Reticulocytes per cent	Nucleated red cells per 100 whites counted		Leuco cytes per c mm	Remarks
						Normoblasts	Megaloblasts		
Average of 27 normal monkeys		6,420,000	91	0 65				18,129	Bilimoria (1931)
Monkey No 7	29–5–30	7,020,000			0 1			18,200	
	8–7–30	5,750,000	84	0 73	0 5			22,000	
	19–8–30	3,850,000	60	0 79	2 0	3		11,200	Blood picture shows anisocytosis (macrocytes) and a little poikilocytosis
	14–10–30	4,020,000	71	0 89	1 5	5	1	13,000	As above plus some polychromatophilia and stippling of reds
	6–11–30	3,528,000	70	1 0	3 0	1		7,800	1st November, 1930 trace of Marmite added to diet Blood picture as above
	13–11–30	4,804,000	79	0 83	5 0	1		23,000	Increase Marmite—stop yeast Blood picture as above
	25–11–30	6,152,000	96	0 80	8 0	1		18,800	do do
	20–1–31	7,110,000	116	0 81	0 2			23,800	Blood picture nearly normal. Some large cells

Monkey No 9	23-1-30	6,200,000	83	0 67	0 1			15,100	
	22-5-30	6,100,000	82	0 67	2 0			19,200	
	20-6-30	5,580,000	96	0 77	0 3			12,500	
	12-9-30	4,918,000	86	0 88	1 0			8,200	Blood picture shows anisocytosis (macrocytes)
	16-9-30								16th to 20th September, 1930, diarrhœa, treated liver extract—extract stopped 21st September, 1930
	26-9-30	3,868,000	54	0 70	2 0	2	1	4,600	Blood picture as above plus some poikilocytosis basophilia and stippling
	14-10-30	3,486,000	51	0 73	0 5	1	0	10,400	No diarrhœa Blood picture as above plus Howell Jolly bodies
	24-10-30	3,558,000	66	0 93	4 0	4	0	10,400	do do
	13-11-30	4,408,000	80	0 91	3 0			9,800	Anisocytosis (macrocytes)
	3-12-30	5,126,000	86	0 83	3 0			11,800	Few large cells Looks well
	22-12-30	6,180,000	102	0 83	1 0			13,000	Still some large cells

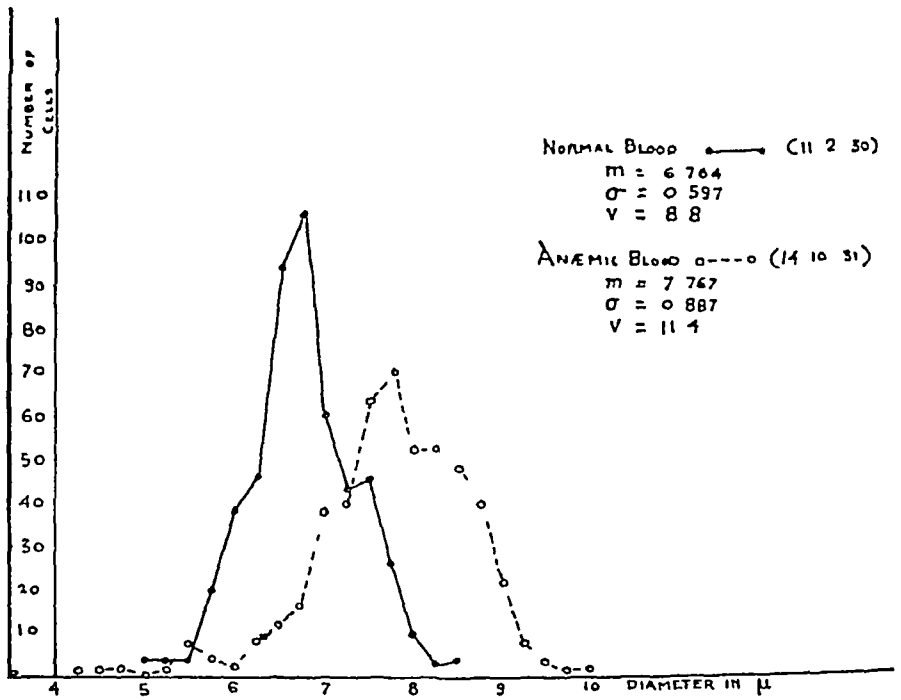
* 5,000,000 R B C = 100 per cent R B C , and 14 g Hb = 100 per cent Hb

The response to Marmite the rapid rise in the red cell count and hæmoglobin percentage and the corresponding increase in the percentage of reticulocytes, was very dramatic in the only case treated (No 7)

Post-mortem examination of the monkeys that died* revealed very striking changes in the bone-marrow There was a marked hyperplasia of the marrow in all the bones and this hyperplasia was undoubtedly of the megaloblastic type

CHART 5

Price-Jones curves, monkey No 7



Plates X and XI show the replacement of fat cells by masses of actively dividing, immature blood cells Apart from fat cells the predominating cell in the slides from normal monkeys is the granulocyte, whereas in the anæmic animals the megaloblast and its precursors are the most obvious feature

DISCUSSION

The experiment reported above shows quite definitely that the feeding of a certain deficient diet to monkeys is regularly followed by the development of a

* Some moribund animals and the controls were shot and examined immediately so that the material was absolutely fresh

macrocytic anæmia in the animals receiving the diet The anæmia resembles very closely that seen in cases of tropical macrocytic anæmia and the marrow picture is that of a megaloblastic hyperplasia The authors were unable to get post-mortem material from their human cases, but assume from the type of anæmia that the bone-marrow must show such a megaloblastic hyperplasia at some stage of the disease The diet which produced this anæmia in monkeys was based on one in common use among sufferers from tropical macrocytic anæmia and was also used in the experiments that led to the production of a macrocytic anæmia in rats (Wills and Mehta, 1930*a*) It would appear probable then that this diet is causally related to the anæmia both in animals and man The diet is not only relatively deficient in all the vitamins (vitamin D was supplied by exposure to sunlight), but also in protein and salts The addition of large doses of vitamins A and C did not protect the animals whereas the further addition of a large dose of Marmite, a rich source of the vitamin B complex and also of good protein and salt value, brought about a rapid cure of the condition The nature of the active fraction cannot be decided from the present work, but it is improbable that it is vitamin B_1, as No 9, although he developed an anæmia, grew well and can therefore be assumed to have had an adequate supply of this factor It is also unlikely that iron is the active curative fraction, as the Marmite used contained only 0 015 per cent iron, which would represent a daily dose of 0 75 mg (0 011 grain) Finally, as Marmite is inactive in the treatment of Addison's anæmia (Wills unpublished, Davidson, 1931) it can be assumed that the curative factor in Marmite is not the same as the anti-anæmia factor contained in liver

But whatever the nature of the anti-anæmic fraction in Marmite, the fact that it is curative, not only in this nutritional anæmia in monkeys, but also in tropical macrocytic anæmia in man, forges another link in the chain of evidence pointing to a common dietetic origin for these two conditions

Summary

1 Monkeys on a diet composed largely of polished rice, white bread and *chapatti*, developed a macrocytic anæmia

2 The bone-marrow in the anæmic animals was hyperplastic, the hyperplasia being of the megaloblastic type

3 Vitamins A and C failed to protect the animals

4 The addition of Marmite (yeast extract) to the diet cured the anæmia

5 One animal, on an additional milk ration, recovered spontaneously

6 The significance of these results is discussed

In conclusion we wish to thank Mr E V Willmott of the Photographic Department, Royal Free Hospital, London, for the microphotographs

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Fig (*a*) Section bone marrow from femur Normal monkey × 310

Fig (*b*) Section bone marrow from femur Anæmic monkey × 310

Fig (*c*) Smear preparation from femur Normal monkey × 575 Price Jones method (1910)

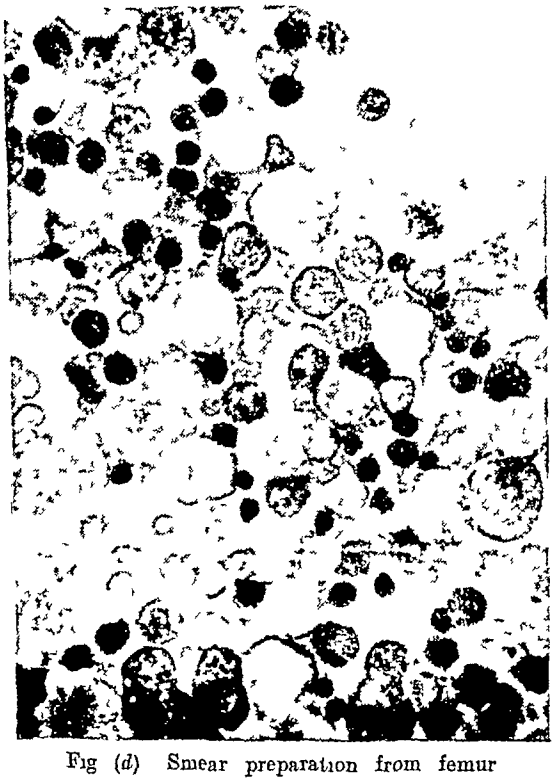

Fig (*d*) Smear preparation from femur Anæmic monkey × 575

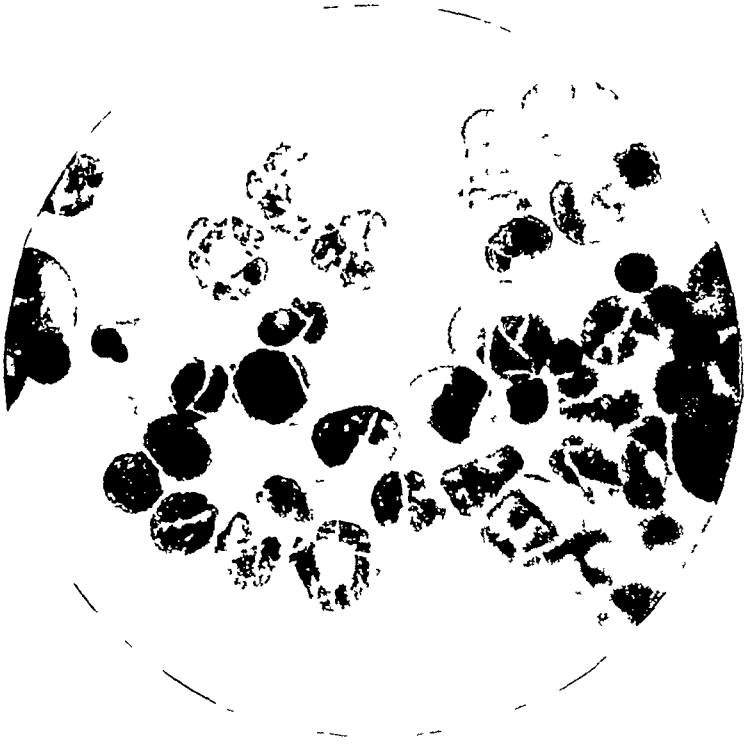

Fig (*a*) Smear preparation from femur Normal monkey × 925

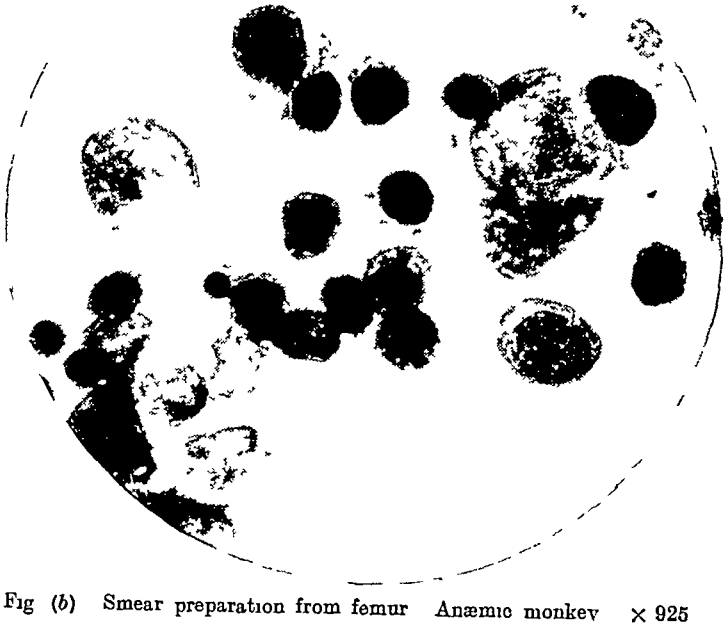

Fig (*b*) Smear preparation from femur Anæmic monkey × 925

THE GENERA *HETERAKIS* AND *PSEUDASPIDODERA* IN INDIAN HOSTS

BY

P A MAPLESTONE, D S O, M B, C H B, D T M

(*From the Hookworm Research Laboratory, School of Tropical Medicine and Hygiene, Calcutta Supported by the Indian Jute Mills Association*)

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INTRODUCTION

FROM the time of Linstow, who made large contributions to our knowledge of the helminthic fauna of the Indian region, a considerable amount of work has been done on the above genera by various workers in India During the past three or four years the writer has accumulated a large collection of nematodes of these genera from birds dying in the Calcutta Zoological Gardens, and this collection has been amplified by examining one hundred domestic fowls purchased in

List of birds examined and parasites found

Species of bird	Number found infected	Parasites found	Number of times found
Tragopan pheasant (*Tragopan satyra*)	9	*H isolonche*	6
		H variabilis	6
		H bosia	6
Silver pheasant (*Euplocamus nycthemerus*)	5	*H putaustralis*	1
		H gallinæ	1
		H papillosa	1
		H isolonche	2
		H pavonis	2
		H parva	1

List of birds examined and parasites found— concld

Species of bird	Number found infected	Parasites found	Number of times found
Ring neck pheasant (*Phasianus torquatus*)	1	*H gallinæ*	1
Kalij pheasant (*Phasianus leucomelanus*)	2	*H gallinæ*	2
		H isolonche	1
Golden pheasant (*Thaumalea picta*)	1	*H gallinæ*	1
Peacock pheasant (*Polyplectrum bicalcaratum*)	5	*H variabilis*	5
Argus pheasant (*Argus giganteus*)	3	*P pavonis*	2
		P voluptuosus	3
		P spinosa	2
Monal pheasant (*Lophophorus impayanus*)	2	*H isolonche*	2
Houbara (*Houbara macqueeni*)	1	*H gallinæ*	1
Sonnerat's Jungle fowl (*Gallus sonnerati*)	1	*H putaustralis*	1
Vulturine guinea fowl (*Acryllium vulturinum*)	1	*H gallinæ*	1
Chakar partridge (*Caccabis chukar*)	3	*H gallinæ*	3
Crested fire back (*Lophura rufa*)	1	*H gallinæ*	1
Coreopsis goose (*Coreopsis novæ hollandiæ*)	1	*H papillosa*	1
Mandarin duck (*Aex galericulata*)	1	? (Females only)	1
Domestic fowl (*Gallus gallus*)	30	*H gallinæ*	30
		H putaustralis	10
		H indica	4
Red-crested wood quail (*Rollulus roulroul*)	2	*P voluptuosus*	2

the local market, thirty of these were found to harbour one or more species of *Heterakis* With the extensive material available the opportunity has been taken of revising these genera as far as they apply to India

The genus *Heterakis* Dujardin, 1845

Heterakis gallinæ (Gmelin, 1790) Freeborn, 1923

This was shown by Cram (1927) to be the correct name of the type species of the genus, which was hitherto known as *Heterakis vesicularis* (Froelich, 1790)

The species is of world-wide distribution and up to the present time it has been recorded from thirty-seven distinct species of birds, which represent several orders, according to the list given by Cram In the present instance it has been recovered from the following birds *Gallus gallus*, *Euplocamus nycthemerus*, *E leucomelanus*, *Phasianus torquatus*, *Thamualea picta*, *Houbara macqueeni*, *Caccabis chukar*, *Acryllium vulturinum*, and *Lophura rufa*

Lane (1917) in his description of this worm, under the name *H vesicularis*, drew attention to the characteristic double curve at the end of the short spicule This curve is only visible when the spicule is seen from the side, and I have found the degree and abruptness of the curve to vary considerably in different individuals (Plate XII, fig 1) The termination of the short spicule of *H longecaudata* is similar, and the only apparent differences between these two species is (so it is claimed by some workers) that the spicules and the diameter of the sucker in *H longecaudata* are somewhat larger than they are in *H gallinæ*

TABLE I *

Comparative measurements of H gallinæ *and* H longecaudata *taken from Cram* (1927) *and obtained by the writer from the hosts shown*

	CRAM (1927)		MAPLESTONE		
	H gallinæ	*H longe caudata*	*G gallus*	*E leuco melanus*	*T picta*
Number of birds infected	?	?	30	2	1
Number of worms measured	?	?	95	5	12
Length, total	7—13	7 9—9 1	5 7—9 1	8 1—9 2	8 0—9 8
Diameter, maximum		0 24	0 26—0 42	0 37—0 38	0 33—0 34
Œsophagus, length				1 0—1 04	0 99—1 41
Œsophagus bulb, diameter				0 22—0 23	0 21—0 24
Sucker, diameter	0 06—0 075	0 08—0 09	0 068—0 104	0 072—0 088	0 078—0 086
Distance of posterior border of sucker from cloaca		0 1—0 15		0 14—0 18	0 16—0 2
Cloaca to tip of tail	0 45	0 42		0 43—0 5	0 46—0 5
Spicules, length	2—2 17 and 0 7—1 1	2 3 and 0 72	1 6—2 5 and 0 73—1 3	2 2—2 3 and 0 65—0 7	2 1—2 6 and 0 6—0 79

* In this and all subsequent tables, the measurements are in millimetres

In all of the above measurements, when a long series was made as in the case of the material from *G gallus*, there was a gradual increase from the smallest to the largest, which did not permit of the separation of one species from another at any given point in the scale Also the increase in size of any special dimension did not depend on the total size of the individuals, for often a relatively large sucker or relatively long spicules were found in one of the shortest specimens, and vice versa It should be noted that the maximum size of the sucker, viz , 0 104 mm , was quite exceptional, and was only found once, in fact only six of the one hundred and twelve specimens measured had suckers over 0 092 mm

Consideration of the above facts together with the measurements given makes it clear that *H gallinæ* and *H longecaudata* cannot be distinguished, therefore *H longecaudata* Linstow, 1879, is a synonym of *H gallinæ* The description and drawings of *H parisi* Blanc, 1913, as reproduced by Cram (1927), suggest the probability that this species is also identical with *H gallinæ*

Heterakis isolonche Linstow, 1906

Baylis (1925) revised Wassink's material and he came to the conclusion that *H neoplastica* Wassink, 1917, is identical with *H isolonche*, and that, as the single male and female in Wassink's collection are exceptionally large, all the dimensions the latter gives are above the average for the species The most definite difference claimed by Wassink appears to be, that in *H neoplastica* the points of the spicules, when extruded a short distance, cross each other, thus differing from the spicules in Linstow's drawing of *H isolonche* Baylis points out that Linstow's figure was probably made from a specimen that had been rolled under the cover-glass to get it into a suitable position, and that the relations of the spicules had been altered by this manipulation In the course of the present work it has been noted that the points of the spicules often do lie across each other when seen from the side, because the long spicule tends to curve ventro-anteriorly and the short spicule tends to curve ventro-posteriorly, but rolling of a specimen even to a slight extent quickly disturbs this relation

Chandler (1926) described *Heterakis lanei* and *Heterakis hastata* both from the crested fire-back (*Lophura rufa*) Chandler apparently bases his diagnosis of these two species on measurements alone, as he does not discuss how they may be distinguished from any previously known species or from each other

The differences in size exhibited in this table between Chandler's two species and the other specimens measured are considerably less than the differences between Wassink's, von Linstow's, and Lucet and Henry's measurements, according to the comparative table given by Baylis (1925), and if this table is combined with mine both of Chandler's species become completely merged in all their measurements between the extremes given From this it seems that both *H lanei* and *H hastata* are identical with *H isolonche* It should be noted that the maximum

TABLE II

Comparative measurements of H isolonche *taken from Cram* (1927), H lanei *and* H hastata *Chandler* (1926), *and the writer's measurements of worms from the birds shown*

	CRAM (1927)	CHANDLER (1926)		MAPLESTONE		
				Pheasants		
	H isolonche	*H lanei*	*H hastata*	Silver	Kalij	Tragopan
Number of birds infected	?	?	?	2	1	6
Number of worms examined	?	?	?	21	7	58
Length, total	7 5—9	9—9 6	10 5—11 5	8 16—9 2	9 4—9 6	7 0—9 1
Diameter, maximum	0 38—0 48	0 29	0 375			0 25—0 42
Œsophagus, length		1 25	1 5			1 0—1 25
Œsophagus bulb, diameter		0 19	0 24			0 16—0 24
Sucker, diameter	0 130—0 150	0 14	0 145	0 90—0 112	0 106—0 108	0 092—0 121
Distance of posterior border of sucker to cloaca		0 175	0 15	0 08—0 12		0 14—0 17
Cloaca to tip of tail		0 4—0 45	0 55			0 45—0 52
Spicules	1 41 (equal) or 1 75 and 1 4	2—2 2 and 2 0	1 75 and 1 65	1 29—1 78 and 1 29—1 68	1 77—1 98 and 1 76—1 90	1 48—2 1 and 0 99—1 37

diameter of the sucker found by me was 0 121 mm whereas the measurements given by other workers vary between 0 13 and 0 15 mm , but Baylis found the diameter of the sucker to vary between 0 09 mm and 0 13 mm , which brings my material within the species

In the case of the material obtained from the Silver and Kalij pheasants the spicules were slightly unequal or actually equal in length, whereas in the Tragopan the differences though very variable were considerably greater on the whole As no other morphological differences could be discovered these differences in relative length of the two spicules are not regarded as significant, especially as Linstow describes them as equal and Lucet and Henry say they are slightly unequal

Heterakis variabilis CHANDLER, 1926

This species was described by Chandler (1926) from the Peacock pheasant (*Polyplectrum bicalcaratum*), and I have also obtained it from the same host and the same locality on several occasions It has been shown by Maplestone (1931), after the examination of a considerable amount of material, that the uncommon arrangement of the caudal papillæ in the male, on which Chandler chiefly based his specific definition, was really an abnormality Further investigation has revealed the fact that this species is also common in the Tragopan pheasant, and the reason it has not hitherto been recognized is that it has probably been confused

with *H isolonche*, for unless careful measurements and also detailed examination of the spicules are made the differences are not apparent Therefore although Chandler based his specific determination of *H variabilis* on a variable character, he happened to name a species not previously recorded so the name must stand

TABLE III

Comparative measurements of H variabilis by Chandler, and by the writer from different hosts

	CHANDLER	MAPLESTONE		
	Peacock pheasant	Peacock pheasant	Tragopan pheasant	Tragopan pheasant
Number of birds infected	1	5	6	
Number of worms measured	?	13	60	28
Length, total	9—10		6 1—7 5	5 9—9 1
Diameter, maximum	0 31		0 25—0 36	0 24—0 45
Œsophagus, length	1 5		0 99—1 45	1 1—1 3
Œsophagus bulb, diameter	0 23		0 16—0 23	0 18—0 26
Sucker, diameter	0 085	0 08—0 096	0 068—0 086	0 068—0 08
Posterior border of sucker to cloaca	0 18		0 13—0 19	0 18—0 24
Cloaca to tip of tail	0 4		0 45—0 51	0 42—0 53
Spicules	1 01—1 88 and 0 72—0 88	1 11—1 24 and 0 73—0 89	1 15—1 68 and 0 69—1 29	1 73—2 33 and 1 48—1 94

Comparison of this table with the measurements of *H isolonche* in Table II shows that there is some overlapping in most points But in the case of the sucker, with very few exceptions all of *H variabilis* are smaller than 0 090 mm , and in no specimen of *H isolonche* was the diameter below this figure In the case of the spicules the length of both is, on the whole, distinctly less in *H variabilis* than it is in *H isolonche*, but there is considerable overlapping as the longest spicules in *H variabilis* are longer than the shortest in *H isolonche* But as with other species examined the longest spicules are not necessarily found in the worms with the other dimensions of the greatest, so combination of a sucker below 0 09 mm with spicules within the range of *H isolonche* is practically always a distinctive point A morphological difference in the spicules was also noted In *H isolonche* the short spicule ends in a simple curve facing caudally when seen from the side, whereas the short spicule in *H variabilis* seen from the same angle exhibits a slight double curve (Plate XII, fig 3) The result of this is that in *H isolonche* the ends of the spicules tend to cross when extruded, as was pointed out by Wassink, whereas the second curve of the short spicule in *H variabilis* prevents them crossing This is of course not an absolute distinction, but it is of considerable assistance in

identifying worms with the spicules extruded and lying on their sides The long spicules appear the same in both species

It will be noted that the specimens from the Tragopan pheasant have been divided into two groups in the table This is because there appear to be two strains of the species, which can be separated by the length of the spicules, there being a gap in the regular sequence of lengths found In the case of the long spicule this comes between 1 68 and 1 73 mm , and in the case of the short spicule the gap is between 1 29 and 1 48 mm , this difference is rendered more striking as the two gaps come together in all specimens It would probably break down if more worms were measured, but this difference is recorded here to indicate that in spite of it there is considered to be only a single species concerned

Heterakis bosia Lane, 1914

I have found this species six times in Tragopan pheasants, and always in company with other species It was originally described by Lane (1914) from the same host It is easily recognized on account of the characteristic shape of the tip of the short spicule, and the relatively great thickness of these organs This is well seen by comparing Fig 4 (Plate XII) with the drawings of the spicules of other species in this paper which are to the same scale

Table IV

Comparison of the measurements of H bosia *given by Lane* (1914), *and made by the writer*

	Lane	Maplestone
Number of birds infected	?	6
Number of worms measured	?	23
Length, total	8 1	8 5—9 8
Diameter, maximum		0 37—0 47
Œsophagus, length		1 3—1 5
Œsophagus bulb, diameter		0 22—0 29
Sucker, diameter	0 25 (0 115)	0 116—0 128
Posterior border of sucker to cloaca	0 35 (0 143)	0 13—0 18
Cloaca to tip of tail	1 3 (0 56)	0 55—0 69
Spicules, length	1 6 and 0 9	1 48—1 85 and 0 87—1 03

It will be noted that my measurements and those of Lane are in fairly close agreement except in the case of the diameter of the sucker, the distance from the sucker to cloaca, and the length of the tail But if these measurements are calculated from Lane's drawing by the scale accompanying it, the figures in

brackets are obtained which bring these three measurements into line with mine for the same points

Heterakis pavonis n sp

In two Silver pheasants male *Heterakis* were found which resembled *H gallinæ* superficially, but the short spicule has a barb similar to that described in *Pseudaspidodera pavonis* Baylis and Daubney (1922) (Plate XII, fig 5) On the whole these worms appear to be smaller than *H gallinæ*, but the point is of little importance seeing the small number of worms available for examination

TABLE V

Measurements of H pavonis

Number of birds infected	2	Sucker, diameter	0 068—0 072
Number of worms measured	6	Posterior border of sucker to cloaca	0 11—0 12
Length, total	6 8—7 2		
Diameter, maximum	0 25—0 27	Cloaca to tip of tail	0 14—0 475
Œsophagus, length	0 91—1 0	Spicules	1 68—1 80 and 0 594—0 673
Œsophagus bulb, diameter	0 11—0 16		

No other morphological differences between this worm and *H gallinæ* could be discovered, except that possibly the notches at the bases of the lips appeared to be slightly better marked, and the dorsal lip appears to be more rounded in *H pavonis*, but these differences are so slight as to be of doubtful value as distinguishing characters (Plate XII, fig 6)

Heterakis indica n sp

During the detailed examination of all the male *Heterakis* obtained from thirty infected fowls, it was noted that in four of the collections there were a few specimens in which the spicules were much shorter than in the smallest of *H gallinæ* Closer examination revealed the fact that the tip of the short spicule was different (Plate XII fig 7) Another point was that the caudal alæ met on the ventral surface anterior to the sucker (Plate XII, fig 8) No other differences from *H gallinæ*, with which they were always associated, could be made out

TABLE VI

Measurements of H indica

Number of birds infected	5	Sucker, diameter	0 064—0 076
Number of worms measured	15	Spicules, length	0 99 —1 19 and 0 297—0 396
Length, total	5 6 —7 2		
Diameter, maximum	0 22—0 28		

Heterakis putaustralis LANE, 1914

This species was described by Lane (1917) from domestic fowls in Bengal, and at the same time he also described *H beramporia* from the same host In his discussion Lane indicates how these two species can be distinguished from *H isolonche H vesicularis* (*gallinæ*) and *H bosia*, but he does not consider how they may be distinguished from each other

TABLE VII

Comparative measurements of H beramporia *and* H putaustralis *by Lane, and specimens from fowls measured by the writer*

	LANE (1914)		MAPLESTONE
	H putaustralis	*H beramporia*	*H putaustralis*
Number of birds infected	?	?	10
Number of worms measured	?	?	25
Length, total	7 6	5 5	4 78—6 34
Diameter, maximum	0 3	0 24	0 18—0 26
Œsophagus, length	0 91	0 745	
Sucker, diameter	0 080	0 055	0 58—0 82
Posterior corder of sucker to cloaca	0 12	0 25 (0 13)	
Cloaca to tip of tail	0 5	0 73 (0 37)	
Spicules, length	0 55 and 0 26	0 7 (0 35) and 0 6 (0 30)	0 32—0 51 and 0 26—0 36

Under the heading *H beramporia* in the above table, where two figures appear, the first one is that taken from Lane's text and the one in brackets is that calculated from his drawings When these corrections are made it is at once apparent that the measurements of Lane's two species come within the variation I have found in my material and which appears to represent a single species

From Lane's descriptions and figures it appears that the spicules of his two species are of different shape His Fig 2 of the spicules of *H beramporia* from the side agrees with my Fig 9 (Plate XIII), and his Fig 6 which is described as the 'left spicule from the venter' of *H putaustralis* agrees with my Fig 10*a* (Plate XIII), which is a ventral view drawing of the spicules from the same specimen that Fig 9 (Plate XIII) was drawn from Lane s Fig 7 which is described as the 'right spicule' is obviously of a lateral view,

as no alæ are visible, whereas my Fig 10 (Plate XIII) shows that both spicules are alate when viewed dorso ventrally My collection of this species was obtained from ten fowls and in the series there is a gradual variation from the smallest to the largest in all the measurements, which does not allow of their separation into two species using size as the criterion It is therefore concluded that *H berampora* is a synonym of *H putaustralis*, priority being given to the latter name as it comes before *H berampora* in the paper in which they are both described

The relatively prominent cloacal region when viewed from the side gives this worm a characteristic appearance, this is shown in Plate XIII, fig 9

In addition to being found in the domestic fowl I have a single male which was recovered from a Sonnerat's jungle fowl (*Gallus sonnerati*)

Heterakis papillosa (Bloch, 1782) in part

Two apparently full grown males of this species, accompanied by two immature females, were obtained from a Silver pheasant In size and general appearance they agree with the description of *H papillosa* by Cram (1927)

Table VIII

Comparative measurements of H papillosa *from Cram* (1927) *and made by the writer*

	Cram	Maplestone
Number of worms measured	?	2
Length, total	9—13	8 4—11 8
Diameter, maximum	0 62	0 35—0 4
Œsophagus, length		1 12—1 3
Œsophagus bulb, diameter		0 18—0 21
Sucker, diameter	0 22	0 216—0 22
Posterior border of sucker to cloaca		0 21—0 30
Tail from posterior papillæ		0 02—0 12
Spicules, length	0 61	0 50—0 54

In the above table the only marked difference in measurements is in the length of the tail, and as this varies in my two specimens between 0 02 mm and 0 12 mm this cannot be regarded as a specific character, especially as the tails are of the same type, viz, a fine point composed apparently wholly of cuticle

Baylis (1923) discussing *H monticelliana* Stossich, 1892, and *H stylosa* Linstow, 1907, disposes of some of the confusion that formerly existed owing to their discordant descriptions In my two specimens in the lateral view, the sucker is not so prominent as in Stossich's drawing (reproduced in Cram), and which Baylis says agrees with his single specimen, but my specimens have a thick cuticular covering to the sucker with a central opening which, if drawn out, would resemble Stossich's figure The papillæ are normal in number and in one of my specimens in which a true ventral view was obtainable there is a pair of sessile papillæ opposite the base of the large post-cloacal papillæ (Plate XIII, fig 11) These are almost certainly a variation from the normal

Baylis (1923) also describes narrow lateral flanges along practically the whole length of the body My specimens agree with this, but it may be added that in the œsophageal region the flanges become broader, forming a distinct elongate cephalic expansion on each side

Heterakis caudata Linstow, 1906

A large number of specimens which I have identified as belonging to this species were obtained from a Coreopsis goose (*Coreopsis novæ-hollandiæ*)

Table IX

Comparative measurements of H caudata *given by Cram* (1927) *and made by the writer*

	Cram	Maplestone
Number of worms measured	?	24
Length, total	7 8	7 3—9 6
Diameter, maximum	0 29	0 22—0 35
Œsophagus, length	0 92	1 3—1 35
Œsophagus bulb, diameter		0 24—0 25
Sucker, diameter	0 18	0 108—0 158
Posterior border of sucker to cloaca		0 18—0 24
Cloaca to tip of tail	0 86	0 32—0 4
Tail from posterior papillæ to tip		0 14—0 2
Spicules (equal)	0 44	0 39—0 51

In the above table the only measurement of mine that does not embrace Linstow's is in the diameter of the sucker, and as this is clearly very variable in this species as well as in all others that have been adequately studied, the difference is not considered significant

Comparison of this collection of worms with the two specimens of *H papillosa* described above, reveals complete morphological identity between them, the only

differences being those of size (Plate XIII figs 11 and 12) For example the praecloacal papillae are relatively far apart in both and the first pair of this group is farther in front of the cloaca than is usual in this genus This character appeared to be constant in all the specimens examined The sucker is covered by a cuticular cup with a central opening attached to the border of the notch in the posterior wall of the sucker The strong radially arranged muscle bands in the region of the sucker are present in both The character of the tail is the same in both The spicules are of the same type in both, with characteristic bistoury-like tips (Plate XIII, fig 13) And finally the expansion of the lateral flanges in the œsophageal region occurs in both (Plate XIII, fig 14) The only differences are thus in size, and these have already been shown to be very variable characters in all the species that have been adequately studied, so they are not considered of importance in the present instance especially as the hosts from which the two collections have been obtained are in different orders viz, *Galliformes* and *Anseriformes* It is accordingly considered that *H caudata* Linstow 1906, is a synonym of *H papillosa* (Bloch, 1782) in part Two other species which show considerable similarity with *H papillosa* according to the descriptions in Cram (1927), are *H brevispiculum* Gendre, 1911, and *H circumvallata* Linstow, 1906, and further study of these species might indicate they are also the same

Heterakis vulvolabita Chandler, 1926

Chandler (1926) described this species from the Black-throated hill-partridge (*Arboricola torqucola*), and I have recovered it from the same host

Table X

Comparative measurements of H vulvolabita *by Chandler and the writer*

	Chandler	Maplestone
Number of worms measured	?	4
Length, total	6—7	5 98—6 5
Diameter, maximum	0 25	0 24—0 28
Œsophagus, length	0 87	0 78—0 8
Œsophagus bulb, diameter	0 165	0 15—0 17
Sucker, diameter	0 05	0 46—0 48
Posterior border of sucker to cloaca	0 09	0 08—0 096
Cloaca to tip of tail		0 28—0 32
Spicules, length	0 535 and 0 29—0 30	0 576—0 600 and 0 312—0 32

These worms agree fairly closely in measurements and, if size alone were the only distinguishing character, it might be thought that they are *H. patanostalis*. But the spicules in *H. calcalbata* differ very markedly in thickness, the longer one being very delicate (Plate XIII, fig. 10) and the shorter spicule also ends in a characteristic tip (Plate XIII, fig. 10), whereas the spicules of *H. patanostalis* are of approximately the same thickness and the shorter one ends in a differently shaped barbed point (Plate XIII, fig. 9).

Heterakis parva Mapletoft, 1921

This species was found in the Silver pheasant on one occasion only, and as no more material is available for study there is at present nothing to add to the description already given.

Heterakis longespiculum Mapletoft, 1931.

This species was obtained from the Red crested wood quail (*Rollulus roulroul*). Since that time a second collection of these worms has been recovered from the same host, and examination of the latter has led me to the conclusion that *H. longespiculum* is identical with *Pseudaspidodera eclupteuses mense* Chandler, 1926. I was originally led into error by not allowing for slight differences in size, and by the fact that in my first collection of this species the cordons on the lips are so indistinct as to be practically unrecognizable as such, but in the second collection these structures are much clearer, thus enabling me to correct my diagnosis.

General considerations regarding male Heterakis.

Throughout this paper it will be noted that no detailed descriptions of the special male characters have been given, with the exception of the spicules. This is because the present extensive investigation has shown that many of the minor points that have been considered of specific value by other workers are not constant in a species, and that examination of a number of worms shows they are subject to great variation.

(1) *The sucker and its related papillæ.* The typical arrangement seen is two fairly long delicate papillæ on each side of the sucker. These may be situated close to the border of the sucker, in some cases (especially the posterior pair) lying in shallow grooves on its outer edge, or the papillæ may lie some little distance from the sucker. There may be a single accessory papilla on one side or a pair of papillæ anterior to the sucker (Plate XIII, figs. 17, 21).

(2) *The precloacal papillæ.* Typically this group consists of four pairs of pedunculate papillæ, and two pairs of sessile papillæ more centrally placed on the ventral surface. Following the numbers in Plate XIII, fig. 17, it is common to find pairs 1 and 3 relatively small and pair 2 the largest, but this varies to some extent and occasionally all four pairs approach equality in size as in any species

examined Another variation occasionally found is that either pair 1 or pair 3 may be missing entirely The sessile papillæ vary slightly in their proximity to or distance from the cloaca, and occasionally a pair of accessory papillæ may be present posterior to the paracloacal group (Plate XIII, fig 17, *A3*) A single pedunculate papilla or a pair may be present between the sucker and the paracloacals (Plate XIII, fig 17, *A2*)

(3) A pair of large pedunculate papillæ are always present between the paracloacal and caudal groups Occasionally there may be a pair of sessile papillæ near the bases of these larger ones (Plate XIII, fig 17, *A4*)

The only male characters which appear to be reliable for specific distinction are shape and, within wide limits, the size of the spicules This applies especially to the shorter spicule in all the species studied, for in practically every instance the tip has a characteristic barb or curve which only varies slightly within the species

Baylis (1925) drew attention to the slight reliance that can be placed on mere measurements in determining species, and this has been abundantly confirmed in the present investigation

General considerations regarding female Heterakis

The females have not been included in the above descriptions for, with one or two exceptions, there appear to be no morphological characters whereby they may be distinguished from each other Thus *H gallinæ, H bosia, H isoloncha, H indica,* and *H pavonis* all appear to be similar The vagina in all of these species is of exactly the same type and it extends forwards in its primary loop for 0 33 mm to 0 79 mm from the vulva It was noted in some specimens that the vagina runs directly forwards from the vulva, and in others it first takes a short bend towards the posterior (Plate XIV, fig 18) Examination of a large number of individuals has led me to the conclusion that this is not a specific character, but the posterior bend arises about the time oviposition begins, and that in young females and gravid worms in which oviposition has not commenced the vagina takes a direct anterior course from the vulva (Plate XIV, fig 18*a*)

The position of the vulva is used by some workers as a specific distinction, but in all the species I have fully investigated I find that the vulva is usually behind the middle of the body length If enough specimens are examined, however, some are always found with the vulva at the middle or in front of it For this reason it is of no value to give the proportions into which the vulva divides the total body length as a specific character

Papillæ in the region of the vulva were described as characteristic of *H isoloncha*, and Baylis and Daubney (1922) expressed the opinion that these were artefacts by the male sucker The present inquiry has amply confirmed this view, illæ' of this nature have been seen in some examples of every species nd their number and position has been extremely variable

TABLE XI

Measurements of female Heterakis

Species of worm	*H. gallinæ*		*H. bonæ*	*H. isolonche* and *H. variabilis*	*H. putaustralis*	*H. papillosa*	*H. indica*	*H. pavonis*
Host	Golden pheasant	Domestic fowl	Tragopan pheasant	Tragopan pheasant	Domestic fowl	Corcopsis goose	Domestic fowl	Silver pheasant
Infection, pure or mixed	Pure	Mixed	Mixed	Mixed	Pure	Pure	Mixed	Pure
Number measured	12	38	20	39	28	10	7	6
Length, total	8 3—10 9	7 9—10 1	9 8—11 1	7 3—10 5	5 08—8 1	8 9—12 5	7 12—7 38	8 8—10 1
Diameter, maximum	0 32—0 39	0 27—0 1	0 26—0 11	0 21—0 37	0 18—0 25	0 2—0 32	0 25—0 32	0 36—0 11
Œsophagus, length	1 06—1 12		1 3—1 51	1 1—1 5		1 15—1 56		1 3—1 1
Œsophagus, bulb, diameter	0 10—0 25		0 22—0 11	0 16—0 26				0 22—0 26
Anus to tip of tail	1 0—1 11		1 2—1 1	1 01—1 1		0 79—1 36		0 91—1 07
Eggs	0 068—0 076 × 0 040—0 042	0 060—0 072 / 0 036—0 040	0 068—0 08 / 0 036—0 01	0 068—0 08 × 0 036—0 01	0 060—0 066 × 0 034—0 038	0 068—0 075 / 0 042—0 048	0 062—0 068 / 0 032—0 036	0 068—0 076 × 0 048—0 044

Lane (1914) in his description of *H. beramporia* describes and figures a vagina and vulva similar to my Fig. 19c (Plate XIV) with a flap covering the vulva from behind. This is the usual appearance seen, but in some specimens the flap over the vulva is anterior or there may be no flap and the vulva opens on a prominence (Plate XIV fig. 19). In his description of *H. putaustralis* all he says in describing the vagina is that '... the cephalad curve of the vagina is complicated'.

This description is not sufficient to distinguish it from any other species and in view of the variability noted in the case of the vulva it is considered that there is nothing in the females of these two species whereby they may be distinguished.

Most of the birds examined had mixed infections as judged by the males recovered from them and all that could be done with the females was to divide them roughly by size, assigning the larger specimens to the species in which the males were larger, but even then many of them came in an intermediate position, and could be equally well classified with either large or small species of males.

With this reservation, the above table has been compiled to give an approximate indication of the size of the females in the various species under discussion. This has been done by choosing where possible, a collection in which males of only one species were present, and where this could not be done collections were chosen in which the males of one species far outnumbered those of any other, and it was then assumed that most of the females present would belong to the species with the males in the majority.

The genus *Pseudaspidodera* Baylis and Daubney, 1922

This genus was erected by Baylis and Daubney (1922) to accommodate *Pseudaspidodera pavonis* which is characterized by the possession of cephalic cordons together with the male characters of the genus *Heterakis*. Only one more species, viz., *P. voluptuosus* Chandler (1926) has since been added to it.

Pseudaspidodera pavonis Baylis and Daubney, 1922

This worm was originally found in the Burmese pea fowl (*Pavo muticus*). I have also found it in this host on several occasions, as well as in a white pea hen (*Pavo cristatus*) and the Argus pheasant (*Argus giganteus*). My examination of numerous specimens has not revealed anything which may be added to the above workers' description.

Pseudaspidodera voluptuosus Chandler, 1926

Chandler (1926) described this species from the Argus pheasant, and a smaller variety from the Red-crested wood-quail (*Rollulus roulroul*). I have found this species in the same two hosts as Chandler recorded it from, and my examination supports his view that the specimens from the quail are smaller than those from the pheasant. There is an additional character which I have noted and which might

be added with advantage to Chandler's description and that is that the primary forward curve of the vagina is relatively long, reaching from 0 9 to 1 4 mm anterior to the vulva (Plate XIV, fig 20)

Pseudaspidodera spinosa n sp

This worm was twice recovered from the intestine of the Argus pheasant (*Argus giganteus*), and on both occasions it was accompanied by *Pseudaspidodera voluptuosus* Chandler, 1926

The mouth is surrounded by three lips, a broad dorsal lip with lateral angles into which long papillæ run, and two large subventral lips (Plate XIV, figs 21 and 22) The cordons may be best considered as three in number, one in relation with each lip and continuous with one another at the interlabial clefts The cordon of the dorsal lip runs posteriorly from the base of the lip on each side and its central portion is recurved forwards to about opposite the end of the first part of the œsophagus (Plate XIV, fig 21) The subventral cordons run a little further back than the dorsal cordon, and they are elongate, simple curved structures without a central re-entrant portion as in the case of the dorsal cordon (Plate XIV, fig 22) The cordons are armed for their whole length by a single row of relatively stout spines

All the other characters are the same as in the genus *Heterakis* so they call for no general description

Male—The caudal alæ appear to be divided into three portions by one transverse groove between the sucker and cloaca and a second one just behind the posterior group of caudal papillæ (Plate XIV, figs 23 and 24) There are twelve pairs of caudal papillæ, the arrangement and relative size of which can be seen in Plate XIV, figs 23 and 24 The spicules are similar but unequal in length

Female—The vulva is a little distance behind the middle of the body The opening itself is not prominent (Plate XIV, fig 25) The vagina begins as a short horse-shoe-shaped muscular tube running forwards, it then bends backwards and running posteriorly, it divides into the divergent uteri some distance behind this opening

This worm has the characters of the genus *Pseudaspidodera*, which may be briefly defined as differing from *Heterakis* in the possession of cervical cordons, and from *Aspidodera* by the presence of caudal alæ in the male It is true that in *P pavonis* the genotype, and in *P voluptuosus* the only other species, the cordons curve outwards and backwards from the inter-labial clefts and do not anastomose with each other, so that there are six short cordons In my species the cordons are very different Similar differences in the cordons in the genus *Acuaria* have led to the establishment of several subgenera, which more recent workers are inclined to make into distinct genera In the present instance, with only three species to deal with, I consider such a subdivision premature, and prefer to include my species in the genus *Pseudaspidodera* under the name *Pseudaspidodera spinosa*

n sp If more species with the characters of this genus are discovered later, a subdivision into subgenera or genera can be effected easily, if the genus is becoming so unwieldy as to render this course advisable

Host *Argus giganteus*

Type-specimens are in the Indian Museum, Calcutta

Table of measurements

	Male	Female
Length	7 8—8 5	9 26—9 46
Diameter	0 15—0 19	0 45—0 49
Cor lons, length		
dorsal	0 2	0 24
subventral	0 22	0 27
Œsophagus, length	0 65—0 69	0 69—0 71
Œsophageal bulb		
length	0 32—0 34	0 32—0 34
breadth	0 26—0 28	0 25—0 28
Œsophagus, anterior part	0 08	0 11
Spicules, length	0 614—0 673 and 0 277—0 317	
Sucker, diameter	0 21—0 25	
Distance between sucker and cloaca	0 14—0 18	
Tail, length	0 39—0 45	1 09
Distance of vulva from tail		3 745—4 058
Eggs		0 060 × 0 036

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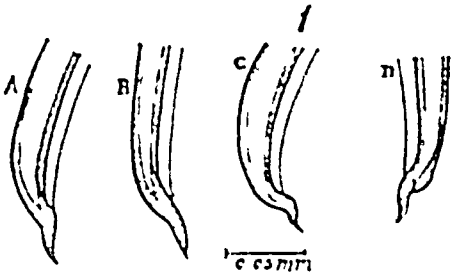
PLATE XII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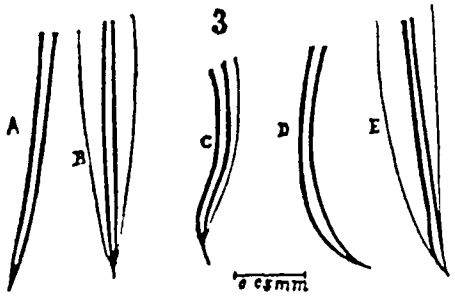
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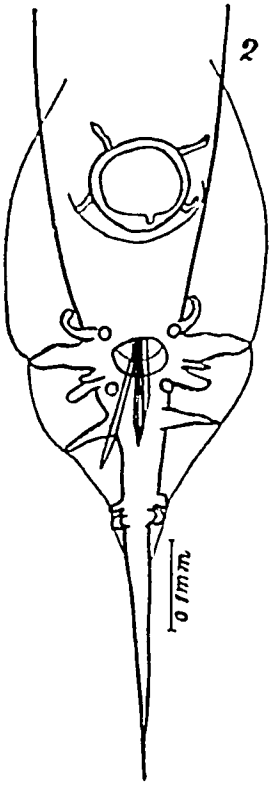
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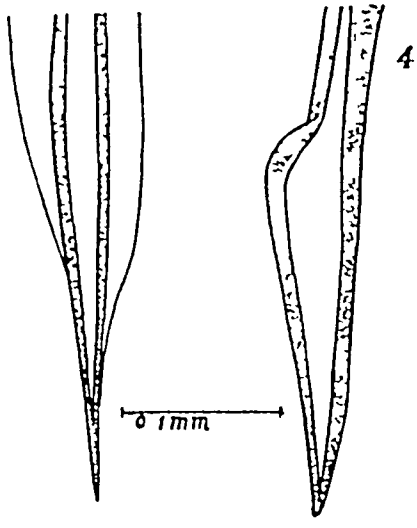
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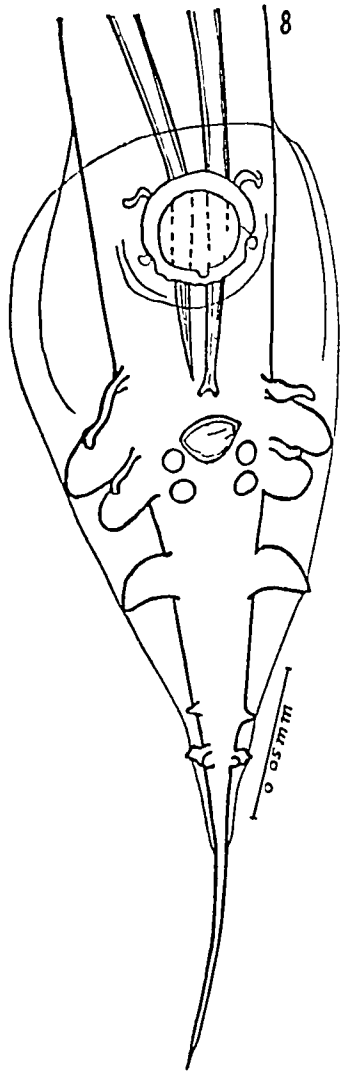
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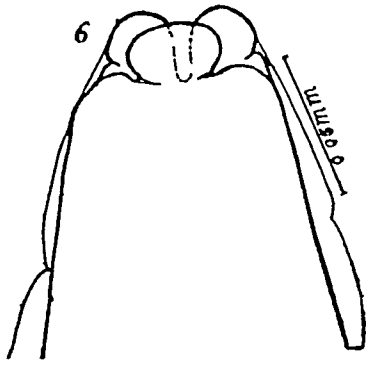
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Explanation of Plate XII

Fig 1 *Heterakis gallinæ* Tip of short spicule, lateral view, showing variation in shape

„ 2 *Heterakis isolonche* Male tail, ventral view

„ 3 *Heterakis variabilis* (*a*) Long spicule, tip (*b*) Short spicule, ventral view (*c*) Short spicule, lateral view

Heterakis isolonche (*d*) Short spicule, lateral view (*e*) Short spicule, ventral view

„ 4 *Heterakis bosia* Tips of both spicules

„ 5 *Heterakis pavonis* Short spicule, lateral view

„ 6 *Heterakis pavonis* Anterior end, dorsal view

„ 7 *Heterakis indica* Short spicule, ventral view

„ 8 *Heterakis indica* Male tail, ventral view

Explanation of Plate XIII

Fig 9 *Heterakis putaustralis* Male tail, lateral view

„ 10 *Heterakis putaustralis* Spicules, ventral view

„ 11 *Heterakis papillosa* Male tail, ventral view (From pheasant)

„ 12 *Heterakis papillosa* Male tail, ventral view (From goose)

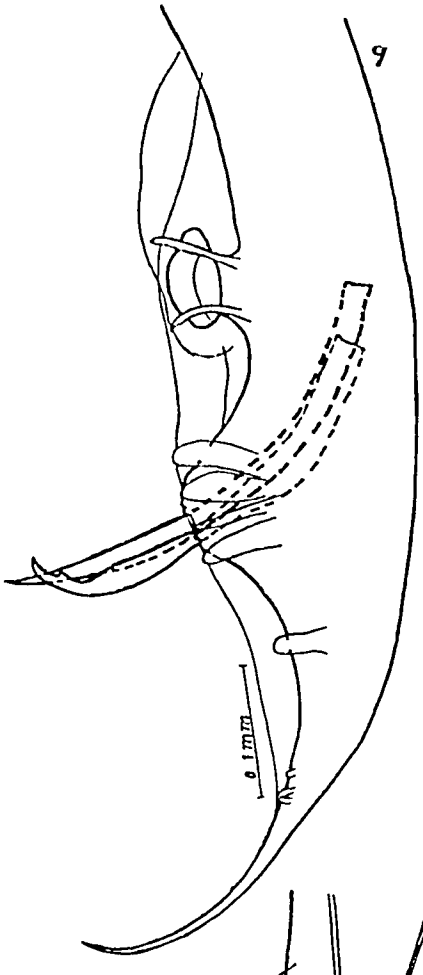
„ 13 *Heterakis papillosa* Short spicule, lateral view, showing variation in shape

„ 14 *Heterakis papillosa* Anterior end, ventral view

„ 15 *Heterakis vulvolabita* Male tail, ventral view

„ 16 *Heterakis vulvolabita* Short spicule, lateral view

„ 17 Diagram of male tail of a *Heterakis* (not to scale)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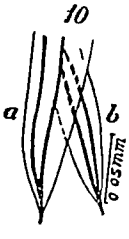
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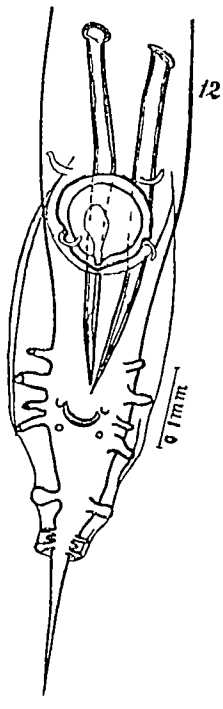
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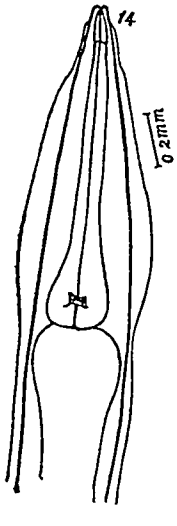
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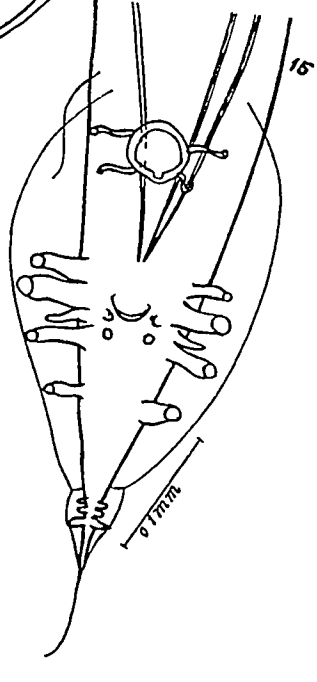
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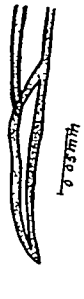
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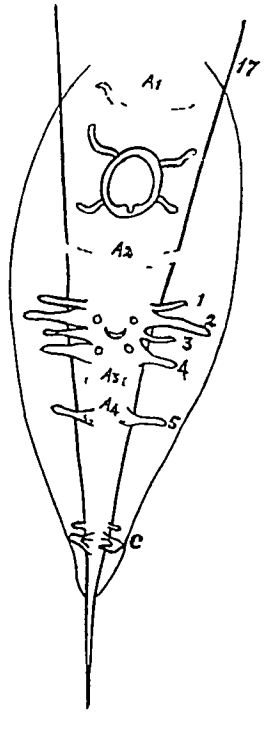
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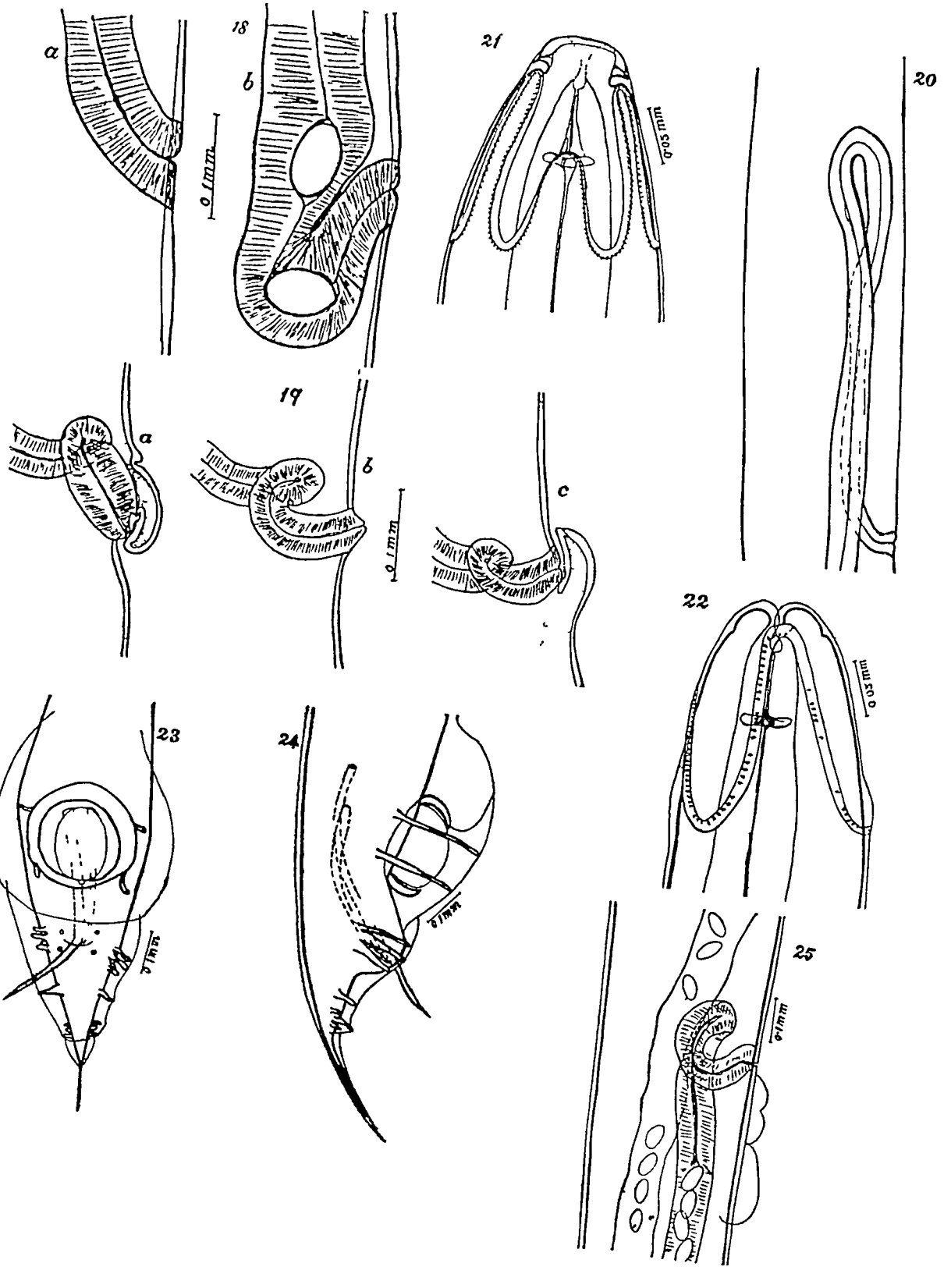
Explanation of Plate XIV

Fig. 18. *Heterakis gallinæ* Vulval region, female
„ 19 *Heterakis putaustralis* Vulval region, female, showing variation
„ 20 *Pseudaspidodera voluptuosus* Vulval region, female
„ 21 *Pseudaspidodera spinosa* Anterior end, dorsal view
„ 22 *Pseudaspidodera spinosa* Anterior end, ventral view
„ 23 *Pseudaspidodera spinosa* Male tail, ventral view
„ 24 *Pseudaspidodera spinosa* Male tail, lateral view
„ 25 *Pseudaspidodera spinosa* Vulval region, female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the actual number and that there were possibly 5 to 10 times as many actual cases

(2) According to census returns and to other information available the incidence varied in different areas It was considered important to find out definitely whether this was so and what were the principal factors influencing the geographical distribution

(3) Racial incidence was another matter to be investigated and the reasons for higher frequency among certain classes of the population

(4) A fourth reason for continuing the survey came to light after it had begun, viz , the local interest created by the results obtained under the first three heads The high frequency of leprosy revealed by the survey called for the training of doctors, initiation of treatment centres, and the appointment of special leprosy officers , in fact local organizations were formed in several of the provinces and states as the direct result of the visits of the survey party

The methods adopted

It was realized from the outset that the only method of carrying out a successful survey was to win the confidence of the people The stigma and odium connected with leprosy make the sufferer from this disease extremely sensitive and timid His tendency is to hide his disease Any show of force was likely to lead to concealment The only inducements which could secure his co-operation were the hope of benefit from treatment, and teaching and persuasion It was therefore by combining propaganda, treatment and survey that we found that results could be obtained , and we called this the P T S (Propaganda-treatment-survey) method Along with this was combined the training of local doctors, some being delegated by local governments and district boards and others attending of their own free will

Usually areas were chosen where local information previously gathered showed that there was the highest incidence and, if possible, other neighbouring areas of low incidence were surveyed to bring out the contrast and compare the conditions

The unit chosen was generally the *thana* or the *taluk* with a population of 20 to 40 thousand Arrangements for a clinic were made in some central and convenient location The villages where cases of leprosy were known to exist were first visited and contacts of infectious cases examined Patients were invited to attend the clinics The interest aroused was often intense An instance of this is shown when during a two months' survey in the South Arcot district the number of patients at the treatment centre went up to 847 From the treatment point of view large numbers are not to be encouraged and such crowds are not likely to last for long or to derive much permanent benefit

especially when they have to be dealt with by an inadequate staff But for the sake of survey and to a certain extent for propaganda the large numbers attending such a clinic are useful, as patients who have attended the clinic can be followed up to their homes and are generally found helpful in bringing other cases to the notice of the survey workers Suspicion and the desire to conceal are removed by the conviction that the doctors are out to help and not coerce them

The party consisted of one chief doctor and four assistants The number of cases it was found possible to examine per week varied according to the density of population, the size of the villages, means of communication and the amount of local support and help that were available In the table the numbers examined, the number of cases of leprosy found and the time required are given

Findings of the survey

(1) *Incidence*—The table gives the incidence in the different districts that were examined Surveys were made in 10 provinces and 4 states Thirty districts and 66 *taluks*, *thanas* or municipal areas were visited Out of 4,560 village areas or municipal wards, 2,536 were found to contain leprous patients Of the 2,435,610 people in the villages visited 16,499 or about 0 7 per cent were found to show clinical or bacteriological signs of leprosy It must, however, be realized that examination of villagers cannot be compared to examination of a labour force or of people who are under discipline and can be lined up and inspected carefully A second visit to a village almost invariably produced several more cases, as the effect of the first visit had been to dissipate suspicion, and those who had concealed their condition were now willing to reveal it The survey figures therefore, though high, cannot be taken as giving the entire incidence in the areas surveyed The only method of finding out anything like the actual incidence in an area is through planting an efficiently run clinic in that area, the doctor-in-charge following up the cases to the villages and examining contacts This would necessarily take years and was quite outside the scope of the present survey In 61 of the districts visited the figures of previous enumerations were available The total cases of leprosy according to these previous figures were 3,414 as compared with 14,893 found by our survey party, more than four and a quarter times as many Again it must be remembered that of these 3,414 cases reported by unskilled enumerators many were doubtless not cases of leprosy at all The result of the survey, therefore, justified the statement previously made by one of the writers that in India the probable incidence of leprosy which can be definitely diagnosed clinically or bacteriologically is not less than between five hundred thousand and a million

TABLE.

Province or State	Districts visited	Number of *taluks* or *thanas* visited	Number of villages surveyed	Number of villages affected with leprosy	Total cases on previous enumeration	Total persons examined by Survey Party	Cases found	Percentage of population showing signs of leprosy	Number of clinics begun	Length of survey in weeks	Castes chiefly affected	Number of doctors trained
Bihar & Orissa	Manbhum	2	544	312	421	168,235	1,525	0 954	2	12	Mahata, Bauri	4
	Santhal Parganas	3	1,003	303	158	121,378	1,080	0 910	3	12	Paharia Santal, Ghatwals Mohammedans	7
	Sambalpur	1	77	45	Not made	43,457	215	0 494	1	2	Ganda, Chamar, Gaur Kumbar	2
	Puri	1	192	143	83	75,857	710	0 935	1	4	Savar, Odapurka Khandait	7
Bengal	Bankura	2	463	345	522	116,853	1,924	1 692	2	8	Kamar Suri Mal, Bauri	3
Burma	Chin Hills	2	198	77	171	79,183	276	0 348	2	8		6
Central Provinces and Berar	Raipur	5	335	187	Not made	173,410	677	0 349	3	4	Chamar, Teli	4
	Drug	1	88	37	do	46,120	119	0 254	1	4	do	1
	Bilaspur	1	52	21	12	36,378	57	0 157	1	4	Teli	1
	Amraoti	1	40	34	Not made	33,154	380	1 146	1	4	Kundi, Mahar, Mali, Mohammedan	1
	Akola	1	90	51	150	42,878	407	0 956	1	4	As above	1
Bombay	E Khandesh	1	113	70	252	103,076	309	0 300	1	8	Kunbi, Mahar Mali	1
	Satara	3	87	70	233	108,027	1,138	0 325	3	8	Marahatta, Mahar, Mang, Dhanger	3

Travancore		2	25	25	93	333,524	1,453	0 431	3	6	Parriahs, Ezhavas, Tandan, Nair	68
Hyderabad (Deccan)		1	112	88	53	60,957	538	0 882	2	4	Mang, Mahar, Dhed, Kumbi	7
Madras	South Arcot	1	156	141	239	190,977	1,909	1 000	1	8	Vaman, Adid ravid, Vellala	8
	East Godavari	3	72	65	438	146,853	1,251	0 852	3	8	Kapu, Mala, Kama, Pan chamas	25
	Madras City		20 (wards)	20	Not made	100,000	206	0 200	3	7	Not noted	41
	Tinnevelly	3	124	90	193	131,116	772	0 588	6	7	do	8
Delhi	Delhi Municipality		16 (wards)	10	3	Not recorded (53 lepers)			1	5	do	2
United Provinces	Barabanki	1	216	144	52	62,140	366	0 588	3	7	Passi, Brahmin, Kurmi	5
	Almora	6	133	66	16	14,240	115	0 807	3	7	Rajpur, Thakur Dome	7
Mandi State		1	47	28	17	2,150	50	2 330	1	4	Girth, Kanait	3
Punjab	Kangra	6	170	60	22	45,712	100	0 313	6	7	do	27
	Amritsar	1	75	5	1	91,480	12	0 131	1	8	Not noted	
Assam	Sylhet	5	18	18	50	16,695	156	0 930	6	4	Bauri, Chamar, Bhagta, Bhumij, Kamar, Chasa	6
	Cachar	4	38	38	73	28,641	228	0 796	20	4	do	20
	Sibsagar	2	16	16	125	28,741	298	1 021	10	4	do	10
	Lakhimpur	3	15	15	40	19,375	157	0 801	8	4	do	8
Central India	Alampur (Holkar State)	1	25	12	7	15,000	62	0 413	1	2	Kerar, Kori, Bania, Basor	1
	TOTAL	66	4,560	2,536	3,414	2,435,610	16,490	0 720	100	178		287

TABLE.

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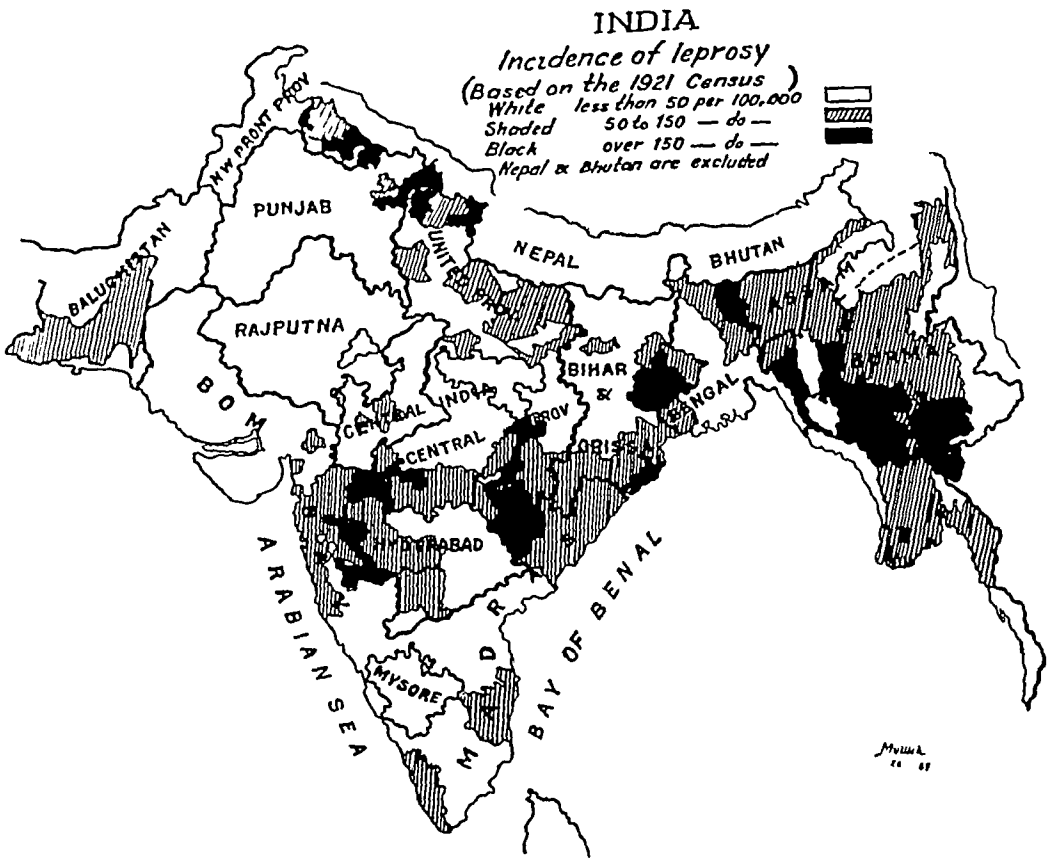
	Amraoti	1	40	34	Not made	33,154	380	1 146	1	4	Kunbi, Mahar, Mali, Mohammedan	1
	Akola	1	90	51	150	42,878	407	0 956	1	4	As above	1
Bombay	E Khandesh	1	113	70	252	103,076	309	0 300	1	8	Kunbi, Mahar Mali	1
	Satara	3	87	70	233	108,027	1,138	0 325	3	8	Marahatta, Mahar, Mang Dhanger	3

(2) The Sub Himalayan districts of Bihar and the United Provinces, the hill districts of the United Provinces and the Punjab and Kashmere Leprosy is also highly endemic in Nepal at least in the central and western parts

(3) The western districts of Bengal south of the Ganges and the neighbouring districts of Bihar

(4) Orissa, and the northern districts of the Madras Presidency which have close Oorya affinities

Map



(5) The eastern division of the Central Provinces (Chattisgarh)

(6) Berar and the cotton-growing central districts of Bombay along with the south-western districts of the Nizam's Dominions

(7) Arcot and Chingleput in the Madras Presidency

(8) Travancore and Cochin

The parts most exempted are as follows —

1 The Ganges valley in Bengal and Bihar

2 The Doab between the Ganges and Jamna in the United Provinces

3 The plains of the Punjab

4 The desert western regions of Rajputana.

5 The Chota Nagpur plateau and the Central Indian plateau extending into the high land of the northern division of the Central Provinces

6 The plains between the Godavari and the Kistna

7 The plateau of Mysore and the elevated surrounding areas

If one seeks for some correlating factors in the more exempted areas one may classify them as follows —

(*a*) Fertile plains between great rivers as between the Godavari and the Kistna, or the Ganges and the Jamna, or in the deltas of rivers like the Ganges

(*b*) Plateaus like those of Chota Nagpur, Central India and Mysore

(*c*) Fertile areas with virile races where wheat is grown and milk is abundant as in the Punjab and Central India

(*d*) Desert areas where the population is sparse as in Rajputana and parts of the Punjab and Central India

On the other hand leprosy is common under the following conditions —

(*a*) In famine areas especially those with a porous, quickly drying soil such as laterite and black cotton As the surface water is low they depend for their crops upon water supplied by the monsoons, any failure or irregularity of which is apt to produce famine conditions This is particularly true of the highly endemic areas in Western Bengal, Eastern Bihar, Chattisgarh and Berar

(*b*) In tracts which have a short time previously been reclaimed from the jungle, and in which accordingly the inhabitants, originally aboriginal, have begun only comparatively recently to mix with the outside world This is specially the case in Chattisgarh, the west of Bengal and the east of Bihar

(*c*) In areas where there are backwaters connected with the sea or river mouths with periodical flooding of a low-lying delta, as in Travancore and as in the bordering districts of Orissa and Madras In these areas filarial infection is very high and possibly the high incidence of leprosy may be partly dependent on this factor

(*d*) In mountainous tracts where the people suffer from privation in the winter months, where the villages are often insanitary and are isolated from one another and from the outside world for a large part of the year Their chief touch with the outer world is through wandering pilgrims (who not infrequently suffer from leprosy) and through some of the villagers temporarily

visiting the plains for trade or cultivation In such isolated villages, once leprosy gains entrance, it is apt to spread among the inhabitants and is carried to other villages through marriage or visits of relatives This accounts to a great extent for the frequency of leprosy in the Himalayas from Nepal to Kashmere

(*c*) In areas rapidly industrialized and into which labour has been imported from highly endemic areas without due safeguards This is true of Assam and of the industrial areas round large cities like Calcutta

Incidence due to racial and social factors

Speaking generally leprosy as it is found in India belongs chiefly to a certain stage of civilization It is uncommon among aboriginal tribes living in inaccessible jungle or hilly districts and following out their tribal laws which, though primitive, are suited to their conditions But when the aboriginal begins to hire himself out as a labourer and thus to come into contact with more civilized conditions in the fertile plains, he forsakes his tribal rules to a certain extent and exposes himself to dangers of infection against which his primitive sanitary laws do not stand proof It is in this semi-aboriginal that leprosy is most common

He is also largely responsible for spreading the disease to the house of his employer and to the higher class employees in industrial concerns Examples of low castes responsible for this are the Pullays and Parriahas in Travancore, the Adidravidas of Madras, the Bauries of Manbhum and Bankura, and the Kahars in the United Provinces

It has been noticed that there are in many places three types of village —

(*a*) that in which there is only one caste,

(*b*) that in which there is more than one, but each caste lives in a separate quarter of the village,

(*c*) that in which there are several castes, high and low, living together, not divided into different quarters

In the first there is little or no leprosy, in the second leprosy is more prevalent, in the third leprosy is much more common

The reason for this is probably that in the first type of village the caste rules are strictly observed, but the more mixing of different castes takes place the more laxity of these rules there is and the greater is the chance of the spread of infection

Movements of population in search of labour

In industrial centres where labour, skilled and unskilled, is congregated within a small area, sexual and other promiscuousness is common and ideal conditions are formed for the dissemination of a disease like leprosy which is spread by

close contact Thus in special leprosy surveys which have recently been carried out in three industrial areas in different parts of Bengal the following incidence was found —

Number examined	Cases found	Percentage
(*a*) 16 880	151	0 9
(*b*) 209 113	2,067	1 0
(*c*) 172 870	2,302	1 3

A doctor working on leprosy in the Western Indian States writes as follows 'People on our side go to Africa and the coal fields (Calcutta) for employment They stay there for 3 to 5 years and about 50 per cent of the cases I found have been infected at these places They have infected their family members and their neighbours and people who come in contact with them, they may be 25 per cent There are 25 per cent remaining who have never gone out, but they have many interesting features and by investigating into the source of infection we can trace that leprosy existed in this district long before'

The above is sufficient to show the danger of the spread of leprosy connected with employment of labour from other parts of India in large industrial concerns This danger may be to a certain extent averted by taking the following steps —

(*a*) Where labour is obtained through recruiting centres, a careful examination of all applicants should be carried out by doctors trained in the diagnosis of leprosy

(*b*) All employees in industrial concerns, not excluding the skilled labour and clerical staff, should be subjected to periodical expert examination

(*c*) It is not sufficient to examine workers alone, as other highly infectious cases (often relations or friends or ex-employees) are frequently found living in the same room with or in close proximity to the workers It is necessary to bring the whole industrial area into the survey and to take means to have all infectious cases carefully isolated, whether they are in actual employment at the time or not

Among other causes of the spread of leprosy which are connected with movement of population may be mentioned —

(1) *Infected markets*—When a market town is highly leprous the infection is often spread to the surrounding villages An example of this was found in the market town of Hiranpur in the Santhal Parganas There the incidence of leprosy was 1 19 per cent Out of the 163 surrounding villages 106 were found to be infected with leprosy Not only this, but people came from most of the districts of Bengal to buy cattle, and the Santals came there to sell their lac So that from a single centre widespread infection may take place

(2) *Pilgrimage* is another source of infection Many of the sacred shrines are infested with persons suffering from leprosy who come either to beg alms or in hope of cure At one temple in South India the god is anointed with sesame oil when he takes his bath in the tank The oil in the tank is believed by pilgrims to cure leprosy, so not unnaturally lepers swarm there, and other worshippers must frequently become infected as all and sundry mix together in the shelters surrounding such places Often the priest himself is found to be suffering from leprosy

(3) *War*—In the Chin Hills the disease appears to have been introduced by captives brought into the country after raids by the wild Chin people on the highly infected plains of Burma

But these are only examples of the many ways in which the movement of population spreads the disease

Dietary errors

Another important factor affecting the incidence of leprosy is the diet of the people Famine is due to geological and meteorological conditions As is mentioned above the porosity of laterite and black cotton soil frequently results in famine when the monsoons fail or are irregular In such tracts also vegetables are difficult to grow and grazing ground is poor The lack of milk and green vegetables results in a condition of the body favourable for the growth of *M lepræ*

In other places such as the Western Ghats high rainfall washes away the soil to the plains below, and the consequent poverty of the land leads to ill-nourishment of the inhabitants Deficient diet is frequently supplemented or made more tasty by the use of decomposing food such as stale rice, fish or meat, and there is reason to believe that such food also predisposes to leprosy

Likewise the food of many town-dwellers is deficient in proteins and fats and vitamins, partly due to poverty and partly to ignorance and social and religious prejudices Highly milled cereals and adulteration of ghee and oil are replacing the more wholesome food of the more primitive village Among Hindus and Buddhists vegetarianism, especially where milk is not available, leads to deficiency in animal protein and fat Correction of dietary deficiencies frequently brings about improvement in leprous lesions even without any special anti-leprosy treatment

The scarcity of leprosy in the plains of the Punjab is probably due, at least to a certain extent, to the comparatively wholesome diet of the people there It does not appear that the people of the Punjab possess any racial immunity to leprosy, as it is fairly common among the Sikhs and others who seek their living in industrial areas in other parts of India

The effects of other diseasfs in predisposing to leprosy

The extent to which the presence of predisposing and accompanying diseases influence the course of leprosy may often be demonstrated by treating these diseases

and observing the gradual improvement of leprous lesions which follows Among the most important of predisposing diseases are malaria, the dysenteries, helminthic infections like ankylostomiasis and filaria, syphilis, typhoid and influenza But any infection which interferes with the general health even to an extent which would not in itself be considered a disease has an important bearing on the course of leprosy

In the highly filarious districts of Travancore, Orissa and Northern Madras leprosy is highly endemic and there is reason to believe that this is not a mere coincidence In the plains of Bengal and in other parts of India perhaps the most important predisposing disease is malaria Patients coming from malarious areas when admitted to the Gobia Hospital and treated for malaria, frequently improve rapidly as regards their leprous lesions without other treatment Leprosy when first introduced into a highly malarious area tends to spread rapidly

Dracontiasis is another disease which has been found to predispose to leprosy In a village in the Satara District of Bombay it was found that leprosy had at one-time been very common At that time guinea-worm was prevalent due to the use of a step well at the lower end of the village The introduction of a closed well at the top of the village with pipes laid on resulted in the disappearance of dracontiasis, and leprosy rapidly diminished also The disappearance of infectious cases was apparently not due to any special efforts at isolation as at the time of our visit we found two cases of leprosy, one, a highly infectious case, was sitting in the midst of the village nursing the child of a neighbour in his lap without any protest from the onlookers In the other the disease had burned itself out and he was no longer infectious, he had, however, unsightly deformities, and he was carefully isolated in a small hut outside the village It should be mentioned here that attempts at isolation of lepers are often made voluntarily by villagers in different parts of India, but it is generally the burnt-out, deformed, harmless case that is isolated, while the infectious cutaneous patient is allowed to mix freely and live inside the village

Ankylostomiasis is another common predisposing disease Examination of the inmates of five leprosy institutions showed an average of 90 per cent infection with hookworm In the largest of these, at Purulia, regularly carried out treatment for this disease improved the general condition of the patients, so that many of those who had formerly made little or no improvement under special anti-leprosy treatment, now began to improve satisfactorily Also the death rate was lowered to a fraction of what it had been formerly

Leprosy is frequently found to follow kala-azar in Bengal (Muir, 1927 and 1927*a*) After the influenza epidemic in 1918-1919 many patients were found who dated the beginning of their leprosy from an attack of influenza In the Santhal Parganas this was found to be markedly the case

The findings of the survey thus tend to show that one of the most important factors in inducing high incidence of leprosy in certain areas is the low resistance of the populace due to other endemic or epidemic diseases This is confirmed by reports from other parts of the world As an example may be mentioned 'The Story of Leprosy in Nauru' (Bray, 1930) in which it is mentioned that leprosy was first introduced into this island in 1912, in 1920 only four cases were known In October 1920, 30 per cent of the population died of influenza, and the general health of the remainder was much depressed by the influenza and dietary deficiency Under those circumstances leprosy increased so rapidly that by 1924 no less than 30 per cent of the whole population were infected

Social and personal habits

Although there is no reason to call leprosy a venereal disease, there is no doubt that sexual promiscuousness has much to do with its spread, especially in industrial areas where public opinion has less hold on the habits of individuals As usual, over-indulgence in food and drink are closely associated evils While it has been stated above that leprosy is chiefly a disease of the lower classes, it is also surprisingly common among the wealthy and among those who hold high positions and are thus tempted to self-indulgence

Rapid spread of leprosy has been noted in villages where due to economic conditions sudden wealth has come to the community It was noted for instance in a village in the Manbhum district, where the sudden demand for lac which arose in the war brought sudden prosperity to those engaged in lac cultivation Not knowing what to do with their wealth they spent it in various forms of indulgence with the consequence that leprosy, already endemic in the village, spread with alarming rapidity

Conclusions

(1) Leprosy has been shown by the survey to be a much more prevalent disease in India than was formerly supposed Probably a million cases would not be an over-estimate

(2) The disease is most common among semi-aboriginals, or aboriginals who leave their tribal seclusion and hire themselves out to agriculturists or to industrial concerns

(3) The infection of other more advanced classes of the community is in the first place largely through the employment of servants in an infectious stage of leprosy Once the infection has entered a family, however, it tends to spread in that family and is often transmitted to friends and acquaintances

(4) Infection with leprosy is probably very widespread and only develops into a clinically or bacteriologically recognizable disease in a fraction of those infected,

the natural resistance of the remainder being sufficient to prevent the disease developing

(5) When leprosy does develop it indicates either hyper-infection, or probably more commonly, lowered resistance This lowered resistance, while it may to a certain extent be inherited, appears, according to the findings of this survey, to be due chiefly to various factors which affect the general health Among these may be mentioned predisposing and accompanying diseases, improper diet, unhealthy habits, climate and surroundings

(6) The movements of population which improved education, transport, etc, have accelerated and made more general in recent years, is a potent factor in the spread of leprosy and in the infection of formerly exempt areas

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INTERIM REPORT ON 'PERNICIOUS ANÆMIA OF PREGNANCY'

BY

A L MUDALIAR, MD, FCOG,

Second Obstetric Physician and Gynæcologist, Government Hospital for Women and Children, Madras,

AND

K NARASIMHA RAO, MB, BS, DGO,

Research Fellow in Medicine, University of Madras

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'PERNICIOUS anæmia of pregnancy', as the name signifies, is a severe form of anæmia which occurs in pregnant women, manifesting itself more often in the later months of pregnancy, and characterized by destruction of red blood corpuscles, a high colour index, and a megalocytic blood picture It may recur in successive pregnancies and is attended with grave risk to the life of the individual, particularly at the time of parturition While this disease seems to be somewhat rare in European countries, it is much more frequent in India and other tropical countries

The incidence of this disease in this country was referred to by one of us (Mudaliar, 1915) in 1915 while working in the Rajah Sir Ramaswami Lying-in Hospital, Madras, in the following terms —

'The anæmia of pregnancy is a malignant type of anæmia that seems to be much more frequent than is supposed, and the experience in this hospital during the past two years has been that it is not only a fairly common complication during pregnancy, but is one of the most fatal complications In 1914, the disease was responsible for 35 per cent of the mortality and in the year under report, 7 out of 17 deaths were due to this condition, giving a percentage of 41 The disease seems much more frequent in multiparæ than in primiparæ, and has a very insidious onset, patients hardly realize the gravity of this condition till the whole body is swollen up and they get an attack of dyspnœa, when they seek admission Breathlessness on slight exertion and extreme weakness, are prominent symptoms An

examination of the blood shows marked reduction in the R B C and a great alteration in their character, variations in shape and size and the presence of nucleated R B C being very common The colour index is high, and excepting for the fact that the polynuclear leucocytes are many lobed—6 or 7 lobes are quite common—no characteristic changes are noted'

In recent times the disease has been studied and described by Balfour (1927) MacSwiney (1927), Mitra (1931), and Wills (1930), since then it has attracted the attention of all obstetricians and general physicians who are interested in lessening the maternal as well as the infantile mortality rates in this country

The incidence of this disease in India is, as has been stated above, much greater than in other countries About 5 to 8 cases in a thousand pregnancies occur in India In Madras during the year 1931 32 cases occurred in the 3 389 confinements at the Government Hospital for Women and Children, while in the United States of America (Oldway, 1931), the incidence varies from 1 in 6,000 to 1 in 10,000 confinements, and it is supposed to be rare in England (Osler, 1919) and Germany (Cornell, 1927) The present paper is a preliminary report on the investigations that are being carried on at the Government Hospital for Women and Children, Madras, on all anæmia cases admitted into the ante natal wards After the examination of the blood, the cases are sorted out according to the hæmatological findings

Secondary anæmias are very frequent in this country, and among the several causes may be stated malaria, ankylostomiasis and syphilis During the year 1931, about 110 cases of anæmia were admitted and on examination, all but 32 of these cases were found to be of the secondary type Out of the 32 cases, 27 had a high colour index, while 5 showed a low colour index, though they presented the appearances of a pernicious blood picture

There is another type of anæmia which we have met with, wherein the blood picture is that of the secondary anæmia with microcytes, the colour index is low, and no megalocytes are found The anæmia is not hæmolytic, but is extraordinarily persistent, and although after pregnancy there is apparently some improvement, a marked degree of anæmia continues The ordinary hæmatinics do not seem to have much effect and these cases do not respond to liver therapy, as do those of pernicious anæmia The cases are associated with hypochlorhydria or achlorhydria

The 'pernicious anæmia of pregnancy' (as we may term the cases of severe anæmia with a hæmolytic picture and a high colour index) differs from the *Addisonian* type in some respects Achlorhydria is not a constant feature, and in several cases varying degrees of hypochlorhydria have been found It is also interesting to note that degenerative changes of the cord are extremely rare, and in the very large majority of cases, even at the time of the crisis, few neurological signs have been observed

PROTOCOL I

Distribution according to race and caste of the 32 cases

	Hindus	Adidravidas	Indian Christians	Mohammedans	Bhuddists	TOTAL
Cases	22	3	2	4	1	32
Per cent	68 75	9 37	6 25	12 5	3 17	100
Total number of admissions	1 703	688	635	83	1	3,389

Amongst the Hindus, it occurs in all castes, both vegetarian and non-vegetarian —

Non-vegetarian	14	43 75 per cent
Vegetarian	18	56 25 ,, ,,

PROTOCOL II

Distribution according to age

	15 to 20 years	20 to 30 years	30 to 35 years	35 to 40 years	TOTAL
Cases	3	23	4	2	32
Per cent	9 35	71 9	12 5	6 25	100

PROTOCOL III

Distribution according to parity

	Primi	II	III	IV	V	VI	VII	VIII	TOTAL
Cases	4	7	7	6	4	2	2		32
Per cent	12 5	87 5							

Distribution in the City—Cases come from all over the City and the suburbs and all parts of the Presidency and no special endemicity has been noted It does not seem to occur in epidemics which is against the view that it is one of the varieties of epidemic dropsy

PROTOCOL IV

Monthly distribution of cases

Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	TOTAL
4	1	2	3	4	7	1		2	3	4	1	32

Wills (1930) in her paper 'Studies in Pernicious Anæmia' observes that 'the disease appears to be seasonal in Bombay, most of the cases occurring between October and March The same incidence is reported by MacSwiney from Calcutta (personal communication) In Bombay, there are several factors such as seasonal variations in the birth rate, the annual exodus to the country and possibly the climatic conditions, which must be considered in relation to the time distribution' In our series, there were 21 cases in the first six months of the year, the maximum number being in June, the hottest part of the year As these anæmias are progressive and they take some months to develop, and since the incidence depends upon and varies with several factors, it is not possible at present to draw any conclusions on this basis, though one is tempted to say that the majority of the cases in Madras occur in the first half of the year, but further observations are required in this direction

Clinical history—Little advance has been made in the clinical observation of these cases and there is not much to add to the clinical description of the senior author 18 years ago (Mudaliar, 1915)

Pernicious anæmia of pregnancy occurs usually between the ages of 20 and 35 It is more common in multiparæ It usually makes itself evident and becomes noticeable between the 5th and 7th months There is often a history of anæmia during or following one or more previous pregnancies The onset is insidious and the patient can rarely assign the exact date of the first beginnings The initial symptoms are unusual weakness, debility, palpitation, and breathlessness on exertion Definite anæmia and pallor is noticed only during the later months of pregnancy Vomiting and diarrhœa are not complained of in every case

Physical signs—The patient is noticeably weak, dyspnœic and presents a fairly lemon-yellow colour suggesting Addisonian anæmia It is neither the muddy

complexion of ankylostomiasis nor the sallow complexion of malaria, but has a distinct feature by itself, a modified lemon-yellow colour The face is puffy and there is œdema of the feet and varying amounts of albumin in the urine The tongue is sore in some cases and there is glossitis in some form or other The appetite is poor The heart is enlarged slightly and hæmic murmurs are present The heart sounds are not rapid, except in the later stages and, if rapid, it is within the limits of the degree of anæmia There is no tick-tack rhythm (which is so characteristic of beri-beri) and no signs of myocarditis to explain the œdema of the feet The blood-pressure is normal or subnormal in some cases Râles may be heard in the lungs, and there may be effusion where there is a severe degree of anæmia Ascites is also common in such cases and the patient complains of scanty micturition Hæmorrhages in the retina were seen in some cases of our series, and the disc is pale The liver and spleen are not noticeably enlarged The condition of the uterus is that found in a normal pregnancy There were no nervous manifestations of the disease in our cases Every case was examined for signs of sub-acute combined degeneration and beri-beri

The disease takes a progressive course until death supervenes, or spontaneous improvement occurs in the puerperium 'Labour generally sets in prematurely and is precipitate the patient gets into a much worse condition, immediately after parturition In fact, this period seems to be much more critical, as with the birth of the child, the breathing becomes more laboured, hyperpnœa and dyspnœa set in, the patient gets into a comatose condition, and although the heart may continue to beat the respirations become more shallow and occur at longer intervals till the patient expires If the patient survives the shock of labour, the prognosis is slightly better, but the first days of the puerperium are still critical days and the slightest indiscretion on the part of the patient leads to a repetition of the above state of symptoms' (Mudaliar, 1915) During labour, there is often a blood crisis, characterized by an increase in the number of megaloblasts, normoblasts, myeloeytes, and reticulocytes in the blood and associated with cyanosis dyspnœa and rapidity of pulse rate

Morbidity and mortality statistics are given in Protocol XI

Hæmatological findings (Protocol X)

(*a*) The blood picture is that of a megalocytic anæmia with a high colour index in the majority of the cases The hæmoglobin varied from 10 to 40 per cent in our cases The R B C is reduced markedly in number, sometimes as low as half million, and nearly all cases showed a count below 2 millions The colour index is high, but it is lower than one would expect in a typical case of pernicious anæmia The blood picture shows megaloblasts, normoblasts, macrocytes, poikilocytes, anisocytosis and less often polychromatophilic punctate basophilia

'In a severe case in a count of 600 leucocytes, there were 294 normoblasts and 27 megaloblasts' Normoblasts and megaloblasts are met with frequently and particularly during blood crises at the time of labour The reticulocytes are found to be about half to one per cent on the average, and in some about 5 per cent even, without treatment The reticulocytic response to liver treatment is similar to that in pernicious anæmia In one case we obtained 25 per cent reticulocytic response on the third day by the injection of Hepatrx P A F (Evans)

(*b*) Leucocytes are normal except that the nuclei of polymorphonuclear leucocytes are many-lobed—6 or 7 in many severe cases

PROTOCOL V

Hæmoglobin percentage, red cell count, and colour index on the average

Series	Number of cases	R B C per c mm	Percentage of Hb	Colour index
Hospital for Women and Children, 1931	32	1 3 million	25 4	1 01

Group distribution of the total red cell counts were made —

1 case showed count of			500,000	per c mm
8 cases	,,	under	1,000,000	,, ,,
5 ,,	,,	of	1,000,000	,, ,,
14 ,,	,,	between	1 and 2 millions	
4 ,,	,,	above	2,000,000	,,

Similarly, the hæmoglobin values range between 10 and 45 per cent and the average reading is 25 4 per cent It is worth while to note here the average figures of sprue-anæmia (Fanley, 1930)

Average R B C count is	374,200
Hæmoglobin percentage	61 7
Colour index	1 0

The difference in the degree of anæmia of sprue and pernicious anæmia of pregnancy is so marked that it is impossible to draw a conclusion that pregnancy anæmia is only a severe degree of 'tropical macrocytic anæmia', if sprue anæmia is to be one of the like

(*c*) *Colour index*—Dare's hæmoglobinometer has been used in all these cases in the estimation of hæmoglobin The average colour index in normal cases was taken as 0 85 The mean colour index was found to be 1 01 showing that average

percentage reduction of hæmoglobin was less than that of corpuscular reduction The results of these are as follows —

1	case	showed	a	colour	index	of 0 6
2	cases	,,	,,	,,	,,	,, 0 75
2	,,	,,	,,	,,	,,	,, 0 8
2	,,	,,	,,	,,	,,	,, 0 9
14	,,	,,	,,	,,	,,	,, 1 0
4	,,	,,	,,	,,	,,	,, 1 1
6	,,	,,	,,	,,	,,	,, 1 2
1	case	,,	,,	,,	,,	,, 1 5

Such a high colour index indicates that this anæmia is a megalocytic type of anæmia However, the Hb percentages are much lower than the average in cases of true pernicious anæmia and sprue anæmia

The *average diameter of the corpuscles* in 'pernicious anæmia of pregnancy' — 'For the estimation of the average diameter of the cells, Eve's halometer (Allen and Hanbury's, London) was used in which 'a thin blood-smear acts as a very efficient diffraction grating and splits up the rays from a small source of light into a brightly coloured halo like a circular rainbow The smaller the particles, the larger the halo and vice versa, so that the large red cells characteristic of pernicious anæmia produce a halo which is smaller than normal'

Twenty cases have been examined thus, and the average diameter is found to be between 4 1 to 4 4 —

1 case	4 4 = 7 81μ
12 cases	4 3 = 8 0μ
6 ,,	4 2 = 8 19μ
1 case	4 1 = 8 39μ

With this instrument, a diameter of 7 6 micron in the dried unstained film is considered to be the upper range of normality Twenty cases examined were found to be megalocytic (7 8μ to 8 4μ)

Gastric analysis in pregnancy anæmia

Different observers reported divergent results Wills (1930) reports from her experience of 50 cases that 'achlorhydria, far from being constantly present, as in the pernicious anæmia is very rarely present in the condition under consideration In one non-pregnant case and three pregnant cases, no free HCl was found, but as the mineral chlorides were above 40 per cent in all cases, the achlorhydria is of a different order to that found in the pernicious anæmia where the chloride values do not exceed 30 to 35 per cent The range of acidity and chloride values is similar to that found in any series of Hospital patients' Paterson, Field and Morgan (1931) report three cases in which free HCl was present We have done only 10 fractional

test-meal analyses and our experience is that there is hypochlorhydria in the majority of the cases and in a few cases there is achlorhydria, which is not different from true pernicious anæmia The chloride content is below normal There is no denying the fact from the analysis as well as from clinical symptoms that there is some gastric dysfunction

PROTOCOL VI

Series	Total cases	Achlorhydria	Hypochlorhydria	Normal	Hyperchlorhydria
Hospital for Women and Children, Madras	10	4	5	1	

We regret we have not done any gastric analysis after histamine injection, since achlorhydria as revealed by the fractional test-meal by itself is by no means synonymous with achylia gastrica Psychical or other reflex causes produce temporary inhibition of secretion and may then cause absence of free HCl We hope to do some more gastric analysis after histamine injections before any definite conclusions are drawn from the above study

In this connection we would like to state that we have observed some cases of simple achlorhydric anæmia which respond to iron therapy The cause of this anæmia is not known, but it is not infrequent in pregnancy and further observations on this subject will be communicated in a later article

The bilirubin content of the serum

All the hæmolytic anæmias give positive indirect van den Bergh reaction In pernicious anæmia, the reading of the units vary from 0 75 to 5 units, the majority being over 1 3 units It is curious in 'pernicious anæmia of pregnancy' the indirect van den Bergh reaction is positive, but the number of units is either one or less than one, generally between 0 5 and 1 5 units This is one of the distinguishing features of pernicious anæmia of pregnancy from true pernicious anæmia Bilirubin was looked for in 20 cases They all are of negative direct reaction The indirect reaction was positive in the majority of cases and the results have been tabulated —

PROTOCOL VII

Series	Direct	INDIRECT			
		Negative	Faint	Less than one unit	More than one unit
Hospital for Women and Children, Madras, 1931 (20 cases)		2	5	17	1

In about 10 cases urine was tested for urobilin and it was found negative

PROTOCOL VIII

Studies in serum, calcium and phosphorus in pernicious anæmia of pregnancy

Series	Number of cases	Inorganic calcium	Inorganic phosphorus
Control	15	9 9 mg per 100 c c	3 4 mg per 100 c c
Anæmia cases	6	8 7 mg average	3 1 mg per 100 c c

The results above recorded show that there is a certain amount of deficiency of calcium and phosphorus and the figures are below normal The same low readings are observed in the estimation of blood-sugar and blood-cholesterol It is no wonder in an anæmic individual that the minerals as well as the body constituents are below normal

Bacteriological studies

The fæces and urine were examined bacteriologically, in the majority of the cases, for any pathogenic organism Anaerobic cultures were made for *B welchii* and *Streptococcus*, and in all cases the result was negative In a few cases *Enterococcus* was isolated in the motion, and *B coli* in the urine of a few other cases There does not seem to be any ætiological relationship between *B welchii*, *Streptococcus* and anæmia of pregnancy or true pernicious anæmia as has been shown by Davidson (1928) in his work on the intestinal flora of pernicious anæmia The results of our series are not far from other observers

Dietetic studies

The diets of the anæmia cases have been found to be in no way different from other normal cases admitted for confinement A careful scrutiny of their diets shows a deficiency of vitamins A, C and D, because of the poor quality and small intake of fats and fresh green vegetables in their diets Amongst the 32 cases there were 14 non-vegetarians and 18 vegetarians In the former there were 4 Mohammedans and 2 Indian Christians and 1 Buddhist Eleven out of 14 non-vegetarians came from better classes There is no lack of protein in their diet (MacSwiney, 1927)

We have not found any vitamin B_1 or B_2 deficiency in their diets nor do the patients suffer from incipient beri-beri or pellagra The disease is met with in all castes and communities The diets in Bombay patients have been analysed by Wills and Talpade (1930) and they also observe that vitamins A and C are deficient

Æetiology of pernicious anæmia of pregnancy

The causation of this anæmia is unknown, but theories concerning its origin are many

Infective theory—The geographical distribution of this disease, in combination with the 'tropical macrocytic anæmia (Wills, 1931), is suggestive of an infective origin The regions affected are India, Malay States, China and the West Coast of Africa We do not know whether this type of anæmia of pregnancy occurs in the areas described The same geographical distribution may explain the vitamin-deficiency theory, because all are rice-eating people in the above areas So far, no causal organism is associated with the disease There is so far no definite seasonal incidence and distribution *B welchii* and *Streptococcus* have not been isolated in any of our cases The work of Davidson (1928) on the intestinal flora in pernicious anæmia and his failure to produce macrocytic anæmia with *B welchii* infection, disproves any infective theory

Vitamin deficiency theory—'Pernicious anæmia of pregnancy' is also supposed to be due to deficiency of vitamins It was thought to be due to A and C deficiency (Wills, 1930) and, experimentally, such anæmia is produced in rats on such deficiency diet, but there is no therapeutic response in these anæmias with A and C vitamin-administration Vitamin B has attracted attention recently and Marmite has been tried in several cases on that score It has not, however, been found to be of use in the 'pernicious anæmia of pregnancy' in our experience, and Davidson (1931) has reported on its failure in pernicious anæmia

It is not out of place to record here the experiments of Guha and Mapson (1931) on the elucidation of the rôle of certain dietary factors in the regeneration of erythrocytes

(*i*) They pointed out that vitamin B_1 deficiency does not produce any reduction in red blood corpuscles by feeding rats on vitamin B_1 deficient food

(*ii*) They produced a reduction in red blood corpuscles count by about one-fifth of normal standard by feeding rats with B_2 deficient diet, but with adequate amount of vitamin B_1 This is overcome by administering autoclaved Marmite which contains only vitamin B_2 But the same response is obtained by feeding these animals with alkalinized, autoclaved liver extract in which practically there is no vitamin B_2 This principle in liver seems to be different from the anti-pernicious

anæmia factor One does not know the nature of the anæmia developed in B_2 deficiency Whether it is macrocytic or not is yet to be studied Apart from it, vitamin B_2 is the pellagra-preventive and if it is deficient in the diets of the anæmic cases, there must be some kind of skin lesion or depilation of hair, which is absent in all the cases It is also noteworthy in this connection that pellagra has not so far been reported in India except in stray imported cases This rules out the theory that vitamin B_2 deficiency is the factor that is responsible for the development of the anæmia of pregnancy The blood picture in beri-beri is not found to be macrocytic in character (Cannon, 1929) Vitamin B_1 deficiency in infants has been studied by Bray (1928) He had not even casually noticed anæmia in their mothers The blood picture of the infants never suggested a pernicious character We have not noticed symptoms of vitamin B_1 deficiency, either acute or chronic, associated with the anæmia There has been no occurrence of epidemic dropsy in recent times in Madras Thus, the vitamin-deficiency theory stands unsupported both by experimental and therapeutic records

Toxæmia theory —Pernicious anæmia of pregnancy is supposed to be definitely a hæmolytic anæmia, probably produced by a toxin from the placenta Schneider (quoted by Ordway and Gorham, 1931) thinks that this is similar to the toxin which causes nephritis and eclampsia When produced early in pregnancy, this toxin is neutralized by the anti-hæmolysin so that little anæmia results Lack of anti-hæmolysin allows the hæmolytic toxin to cause undue destruction This, though plausible, does not seem to help us materially in correctly understanding the pathology of the anæmia, in all cases There is no high blood-pressure, and there is no retention of non-protein nitrogen

Deficiency of anti-anæmic factor —Alder (quoted by Ordway and Gorham, 1931) considered this anæmia as a reaction of previously damaged bone-marrow to the unusual demands of pregnancy In the pernicious anæmia of pregnancy, we see both —

(1) Increased destruction, and (2) Deficient blood production

(*a*) It has been proved that there is some degree of physiological anæmia in every pregnant woman (Gullaways, 1929) There is increased blood destruction in a pregnant woman, so that the iron may be made available for the fœtus, to be useful not only for the intra-uterine existence but also for the first six months of the extra-uterine existence The recent work of MacKay (1931) on the nutritional anæmias of infants and the work of Kotekoff (1931) on the iron of blood in infants, prove that the infant largely depends on storage of iron in the liver for the first six months of its life The iron in milligrams at birth is 61 8 and the lowest that is recorded is at the 9th month—39 7 mg Thus, we see that the mother has a double function in the general metabolism

as well as the mineral metabolism, such as iron, calcium, phosphorus, etc Normally the mother gets all these elements from the diet and the metabolism of each is regulated to such an extent that there is greater absorption and less excretion However, there must be increased supply of the elements in the diets The iron in the organic form that is obtained from the destruction of red blood corpuscles in the mother is passed on through the placenta and it is more easily utilized by the fœtus *in utero* The increased blood destruction is compensated by increased blood production and it normally passes off as a hypochromic physiological anæmia of pregnancy with a lower Hb percentage and lower red blood corpuscles count than normal So long as the hæmapoietic system is able to balance the increased destruction and the extra demand of the fœtus there will be no sign in the blood picture of any pathological manifestation The bone-marrow need not necessarily be damaged but its threshold may be low, so that its powers of regeneration are limited, as the substances that stimulate regeneration are deficient

(*b*) *Gastric derangement and megalocytic anæmia*—Castle (1930) has shown that normal gastric juice contains substances which elaborate from beef muscle another substance that promotes normal blood regeneration, but that this power is absent from the gastric secretion of patients suffering from pernicious anæmia The first substance elaborated in the course of the digestion is called the Intrinsic Factor, the second, the Extrinsic Factor, and the third, the Anti-anæmic Factor Castle's work proves that the absence of anti-anæmic factor underlies the megaloblastic hyperplasia of the bone-marrow in pernicious anæmia The anti-anæmic factor is stored in the liver, kidney, etc, and it is necessary for the maturation of the red blood corpuscles from the megaloblast to the megalocyte or to the erythrocyte, even in the pernicious anæmia of pregnancy as in true pernicious anæmia Both from clinical observation and fractional test-meal, we find in pernicious anæmia of pregnancy that there is some derangement of the stomach function It should, however, be noted that achlorhydria, hypochlorhydria or achylia gastrica are only accompaniments of some more ultimate gastric defect which in itself is the cause of the disease both in true pernicious anæmia as well as pregnancy anæmia

We think that there are three factors in the causation of the disease —

(1) Constitutional factor, (2) A factor associated with pregnancy, and (3) Deficiency of anti-anæmic factor

The possible hypothesis in this anæmia is that there is normal or low secretion of the intrinsic factor which is enough in a normal individual and it is not enough in a pregnant woman That the mother supplies the fœtus all the nutrition in the form of end products and that there is no digestion in the infant are well known The anti-anæmic factor that is required for the maturation of the fœtal red blood

corpuscles is supplied by the mother It is our experience to see infants born of these anæmic patients having normal hæmoglobin percentage values (Protocol XII) and normal red blood corpuscle counts and normal blood picture, though they may be premature The mother has a double demand Thus, the mother supplies the infant at her cost, all the materials including the anti-anæmic factor The maternal hæmopoietic system suffers, resulting in the anæmia with abnormal formation of red blood corpuscles which are more easily destroyed by the reticulo-endothelial system In such constitutionally predisposed individuals, the amount of anti-anæmic factor is deficient and hence this megaloblastic blood picture and megaloblastic changes in the bone-marrow This explains why the post-mortem appearances resemble those of true pernicious anæmia

Rational therapeutics in 'pernicious anæmia of pregnancy'

Pernicious anæmia of pregnancy is essentially a deficiency disease—a deficiency not of vitamins, but of the intrinsic factor necessary for the elaboration of the proper amount of the anti-anæmic factor in the gut by a process of digestion This deficiency has to be combated by administering this factor in the form of liver, which contains it abundantly, or the intrinsic factor in the form of hog's stomach extract (Ventriculin) as suggested by Sturggis (1929) Early treatment must be instituted as the disease often ends fatally as is seen by the morbidity figures, and it should be the aim of the obstetrician to raise the blood count to at least 3,000,000 and Hb percentage to 50 before term The large amount of vitamin-content in liver created a great enthusiasm that vitamins are the primary factors in the regeneration of the red blood corpuscles in the anæmia of pregnancy The essential treatment may be summed up as follows —

1 The institution of proper diet and rest
2 The treatment of the megalocytic anæmia with liver or Ventriculin
3 The reinforcement of demonstrable deficiency by such means as HCl, iron, vitamins A and C
4 Transfusion of blood in urgent cases

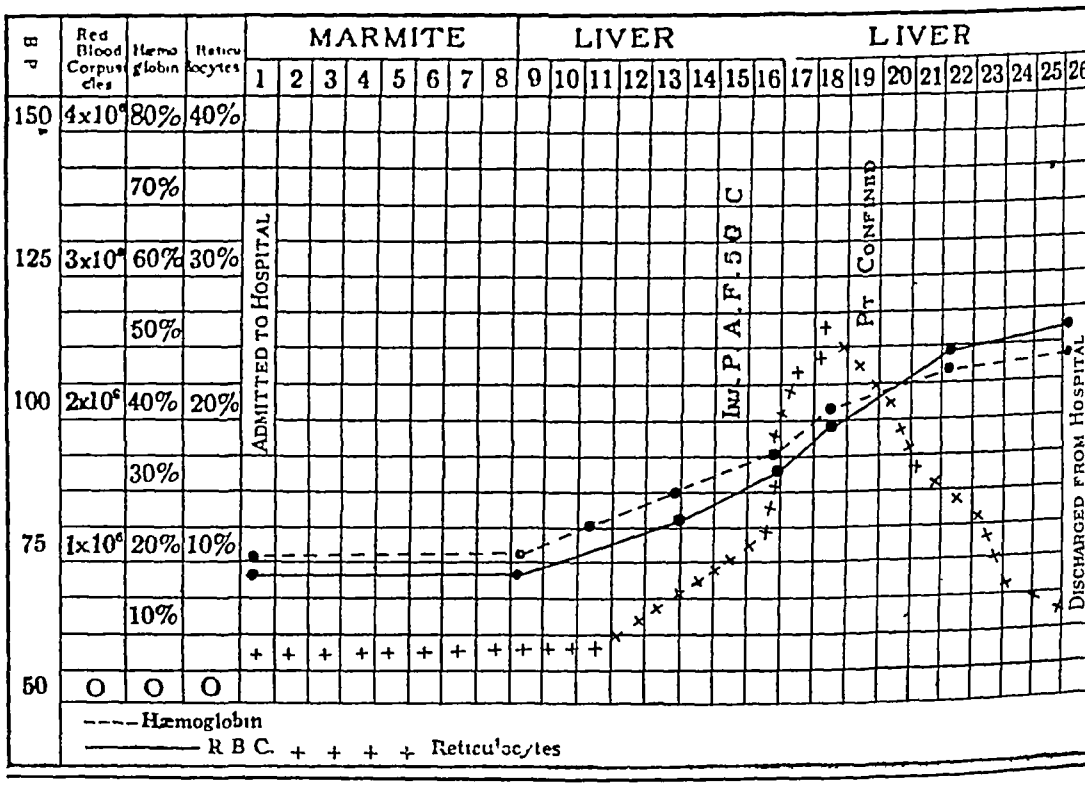
The treatment must be adopted to the individual requirements of the case and where concomitant diseases such as malaria, ankylostomiasis and syphilis exist, specific therapy must be instituted

Liver treatment in 'pernicious anæmia of pregnancy'

The epoch-making discovery of Minot and Murphy (1927) of liver as a cure for pernicious anæmia has changed the treatment of pernicious anæmia of pregnancy also Liver is found efficacious in all megalocytic anæmias as is shown by Vaughan (1930) and others Wills (1930) and Balfour (1927) have shown that the response to liver therapy is just the same as in pernicious anæmia, though a little slower The

response to liver treatment has been observed carefully in several cases in this hospital In our experience, as also of others (Keefer and Yang, 1931), the administration of liver and iron has been of great value in this anæmia (Charts 1, 4 and 5) The deficiency of iron has also to be taken into consideration Infection, inadequate dosage, administration for too short a period, and administration in the secondary anæmia cases, are the causes of the so-called failure of liver therapy

HÆMATOLOGICAL CHART 1



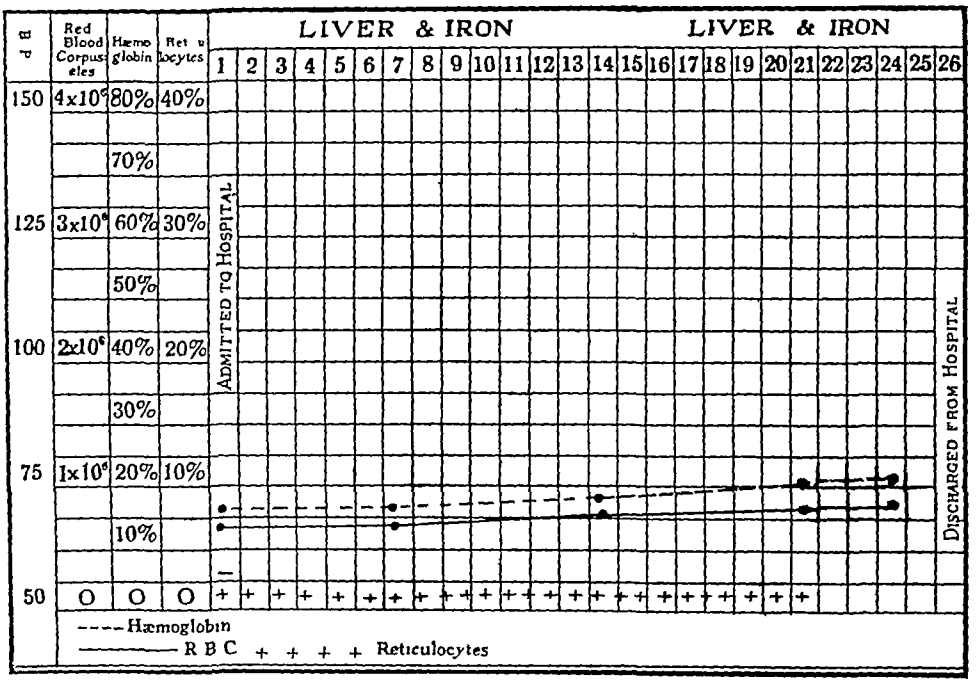
No 18 —Case of pernicious anæmia of pregnancy, V-para Treated with Marmite for 8 days From the 9th to 26th day liver was given On the 15th day P A F (Evans) 5 c c was injected Response to liver therapy Patient confined on 19th day (premature delivery, baby, weight 5 lb) Discharged on the 26th day

The reticulocytic response is similar to that observed in Addisonian anæmia, the reticulocytes reaching a maximum between the 5th and 14th days and then falling down

Ventriculin was tried in some cases and it has been found to be equally efficacious, showing that the essential cause of the disease is the lack of or the deficiency of the intrinsic factor.

In a very bad case, Hepatix P A F (Evans) was administered intramuscularly and found to be very encouraging (Chart 1) It is worth while noting here that Hepatix P A F contains very little vitamin-content and the response of this disease to this extract shows that the essential cause is the deficiency of the anti-anæmic factor and not the vitamins Though liver contains vitamins B_1 and B_2 in excess (Guha, 1931, 1931*a*, Gilroy, 1931), they do not help in the regeneration

HÆMATOLOGICAL CHART 2



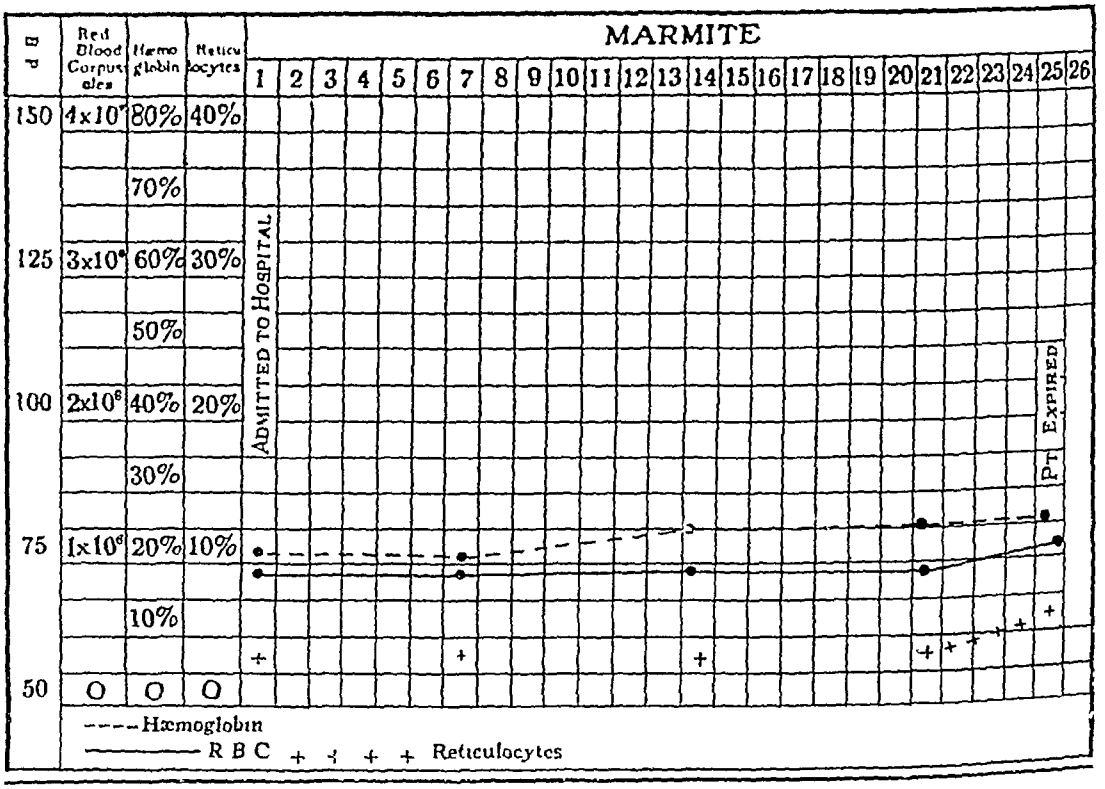
No 19 —Case of pernicious anæmia of pregnancy, VII para, 9 months Was treated with Marmite for 15 days with no appreciable improvement Later, started on liver and iron Treated for 21 days with liver and iron No improvement No reticulocytic response Failure of both liver and Marmite

of the blood in any way except by improving the general health and increasing the metabolism

The dosage of liver must be adequate About ½ lb per diem must be given But in cases with severe albuminuria, liver is not given in such large doses as it necessarily increases the protein intake In such cases the Hepatix P A F seems to be the only rational method of administering the specific factor

Vitamin treatment—Interest in this form of treatment was roused by the contribution of Wills (1931) on the effect of Marmite in the treatment of pernicious anæmia of pregnancy. The clinical improvement according to Wills (less œdema, easier breathing, return of appetite) was found by the 3rd day and the improvement in blood condition on the 4th day. Marmite has been tried in about

HAEMATOLOGICAL CHART 3



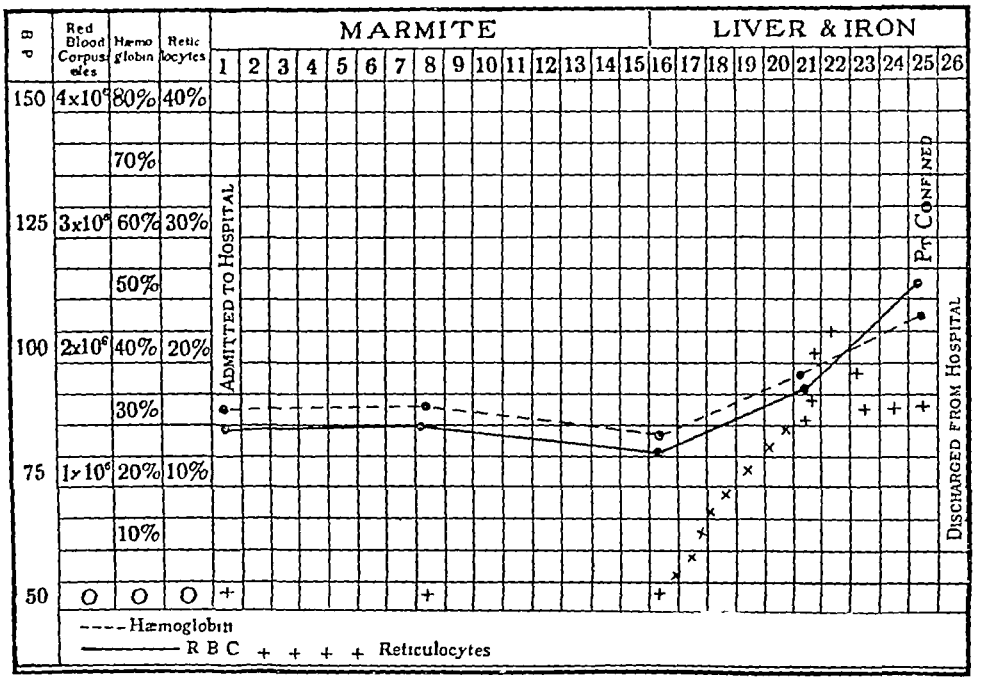
No. 20.—Case of pernicious anæmia of pregnancy, IV-para, 7 months pregnancy. Treated with Marmite from the 1st day. Patient got into labour on the 25th day. Blood crises. Numerous megaloblasts, normoblasts, reticulocyte 5 per cent. Expired soon after.

20 cases in all by us. Eleven cases were typical pernicious anæmia of pregnancy (Protocol IX) and the rest were secondary anæmia cases. The results were found to be disappointing (Charts 1, 3 and 4). The case reports are given below. The reticulocytic response was not found in any of our cases. We regret we have not been able to confirm the results of Wills in the treatment of pernicious anæmia of pregnancy with Marmite.

Reinforcement of demonstrable deficiency by such means as HCl, iron, vitamins A and C—If there is achlorhydria, or hypochlorhydria, it is undoubtedly a reasonable procedure to administer HCl as pointed out by Hurst—half to one drachm of acid hydrochloride dil given in dilute orange juice or water at the end of a meal

Iron is found to be a useful adjuvant to liver and in cases with such low Hb values, it is always better to continue iron with liver therapy (Charts 4 and 5)

HÆMATOLOGICAL CHART 4



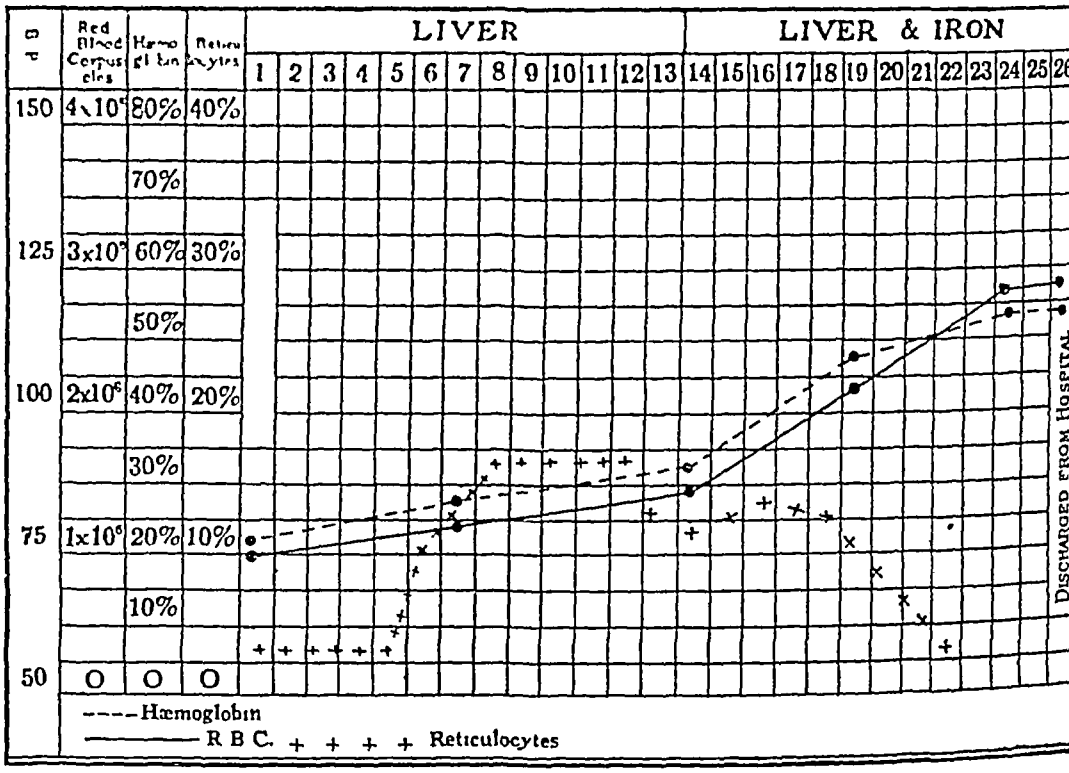
No A (Extra series), 1932—Case of pernicious anæmia of pregnancy, IV para Treated with Marmite for 15 days with no apparent improvement Started on liver and iron on 16th day Improvement with reticulocytic response Patient confined on 25th day (Baby, weight 6 lb)

These charts show the response of the patients suffering from pernicious anæmia to Marmite, to liver therapy and to liver therapy with other adjuvants Chart 2 shows the failure of liver therapy as well as Marmite

We are continuing our work in this direction for which the Indian Research Fund Association has kindly given one of us (A L M) a grant from 1st April,

1932, and we hope to discuss this important and interesting subject more in detail in our next contribution

HÆMATOLOGICAL CHART 5



No 25 —Case of pernicious anæmia of pregnancy, II para, 8 months Severe albuminuria Liver therapy started (on the 12th day after admission when albumin in the urine was traced) On the 14th day of liver therapy the patient was put on iron in addition Response much greater Patient confined on the 25th day of treatment Discharged cured subsequently

SUMMARY

Pernicious anæmia of pregnancy is much more common in tropical countries, it is a severe form of anæmia characterized by destruction of the red blood corpuscles, a high colour index and a megalocytic blood picture The incidence of this anæmia is discussed under various heads, age, race, parity, and seasonal distribution, with particular reference to Southern India The clinical picture of the disease has been described The hæmatological, biochemical, bacteriological and gastric analyses have been given for the cases that were studied in this hospital

It is suggested that three factors enter into the causation of the disease—the constitutional factor, the factor associated with pregnancy and the deficiency of the anti-anæmic factor

'Pernicious anæmia of pregnancy' is a definite entity by itself It differs from the Addisonian type in the absence of neurological symptoms in the large majority of cases, the presence of some degree of hypochlorhydria and the marked crises that occurs particularly at the time of labour

From the treatment point of view, it has been found that this anæmia responds well to the various preparations of liver, that Marmite, a preparation of vitamin B, has not been found to be of much use in the treatment of this condition, that a combination of liver with such hæmatinics as are usually given for secondary anæmia, is probably better in the large majority of cases, that there are certain cases of pernicious anæmia of pregnancy which respond to a degree to liver therapy, but do not show marked improvement after a particular stage, that there is a type of secondary anæmia with no definite cause and with achlorhydria which responds less to liver therapy and which requires further investigation

The morbidity and mortality in the series of cases investigated are given in Protocol XI

PROTOCOL IX

Marmite treatment in pernicious anæmia of pregnancy

Case number	INITIAL READING		END RESULT		Duration of Marmite† medication in days
	R B C in millions	Hb per cent	R B C in millions	Hb per cent	
3	0 9	21	1 0	23	8
10	1 1	24	1 5	28	15
11	1 35	28	1 24	30	23
12	1 18	20	1 0	20	18
13	1 5	30	1 14	30	16
17	2 2	44	2 4	45	18
18	0 9	20	0 9	21	8
19	0 8	20	0 8	20	15 (No improvement with liver also later)
20	1 0	20	1 0	23	24
33*A	1 5	30	1 0	22	15
*B	1 9	45	1 8	45 5	21

* 33 A and B are not cases of this series but of 1932 † Dose 1 drachm three times a day

Protocol X

Blood counts, blood picture, treatment and results of cases

Case number	Condition when count was taken	R B C in millions	Hb per cent.	Colour index	Leucocytes	Halometer	Blood picture.								Treatment given	Duration of treatment	Result
							Megalocytes	Megaloblasts	Normoblasts	Reticulocytes	Poikilocytosis	Anisocytosis	Polychromatophilia	Punctate basophilia			
1	A N and M	1 0	20	1 0	5,000		+	+	+	2	+	+	+	—	Liver	14 days	Patient discharged cured, baby expired
2	M	0 9	20	1 2	4,700		+	+	+	1	+	+	+	—	No liver		Premature 4 lb Discharged alive
3	A N	0 9	21	1 1	6,000		+	—	+	1½	+	+	+	+	Marmite for 8 days	8 days and later liver	Discharged alive No improvement with Marmite
4	A N	1 05	20	1 0	6,000		+	+	+	½	+	+	+	+	General treatment.	4 days	Patient expired baby premature 2¼ lb
5	A N	1 26	25	1 01	12,000		+	+	+	2½	+	+	+	+	General treatment	1 day	Patient expired baby premature 3 lb
6	A N	1 25	25	0 8	2,000		+	+	+	½	+	+	+	+	Liver	3 months	Mother and child alive, discharged cured
7	A N	0 7	18	1 2	4,000		+	+	+	2	+	+	+	+	Liver	2 months	do
8	A N	0 9	20	1 2	5,000		+	+	+	1½	+	+	+	—	General treatment		do.
9	A. N	1 3	28	1 2	4,000	4 3	+	+	+	1	+	+	+	—	General treatment and liver	35 days	Premature, still born, mother alive
10	A N	1 1	24	1 01	6,000		+	+	+		+	+	+	—	Marmite	15 days	No improvement premature still born mother alive

11	A N	1 13	28	1 5	5,000	4 3	+	+	+	1	+	+	+	+	Marmite	23 days	No improvement still born baby, premature, mother alive
12	A N	1 18	20	0 9	6 000		+	+	+	1	+	+	+	—	Marmite	18 days	No improvement premature still born baby, mother alive
13	A N	1 5	30	1 0	4,700		+	—	—	1	+	+	+	—	Marmite	16 days	Premature, alive, mother alive no improvement
14	A N	1 88	39	1 12	5 200		+	+	+	½	+	+	+	—	Liver		Premature, mother alive
15	A N	0 98	20	1 01	4,900	4 2	+	+	+	½	+	+	+	—	General treatment		Patient expired
16	A. N	2 2	45	1 01	5,200	4 2	+	—	—	1	+	+	+	—	Liver	26 days (improved)	Natural delivery mother and child alive, discharged cured
17	A N	2 2	44	1 0	6,000	4 3	+	—	—	½	+	+	+	+	Marmite	18 days	No improvement mother and child alive
18	A. N	0 9	20	1 1	6 000	4 2	+	+	+	½	+	+	+	+	Marmite / Liver	8 days / 14 days	Premature, 5 lb, slight sapræmia
19	A N	0 8	20	1 2	4,000	4 2	+	+	+	½	+	+	+	—	Liver and Marmite	2 months	No improvement premature, 5 lb, mother alive
20	A N	1 0	20	1 0	7 000	4 1	+	+	+	3	+	+	+	+	Marmite	24 days	No improvement patient expired, macerated fœtus
21	A N and M	1 7	35	1 02	6,500	4 4	+	+	+	2	+	+	+	—	Liver	6 days	Premature
22	A N	1 78	40	1 12	6,000	4 2	+	+	+	4	+	+	+	+	Liver	19 days	Improved operated for secondary abdominal pregnancy patient expired of shock
23	A N and M	2 0	50	1 2	4 500	4 2	+	—	—	2	+	+	+	A few	Liver	6 days	Natural
24	A N and M.	1 1	22	1 0	5,000	4 3	+	+	+	1	+	+	+	—	Liver	60 days	Improving natural, discharged cured
25	A N	0 9	20	1 1	6 000	4 3	+	+	+	5	+	—	+	+	Liver and iron	28 days	Improved still born sapræmia

Protocol X—*concld*

Case number	Condition when count was taken	R B C in millions	Hb per cent	Colour index	Leucocytes	Halometer	Blood picture								Treatment given	Duration of treatment	Result
							Megalocytes	Megaloblasts	Normoblasts	Reticulocytes	Poikilocytosis	Anisocytosis	Polychromatophilia	Punctate basophilia			
26	A N	2 15	38	0 9	4,000	4 3	+	+	+	1	+	+	+	A few	Liver	Few days	Premature 2¼ lb
27	A N and M	1 06	12	0 6	4,000	4 3	+	+	+	½	+	+	+	A few	General treatment	1 day	Patient expired after giving birth to a premature baby
28	A N and M	1 0	15	0 75	6,000	4 3	+	+	+	¼	+	+	+	—	Liver	Few days	Premature, 2¼ lb
29	A N	0 5	10	1 0	4,000	4 3	+	+	+	2	+	+	+	+	Liver	13 days	Improved premature, 4 lb, died next day, mother alive
30	A N and M.	2 0	30	0 8	4,000	4 3	+	+	+		+	+	+	+	General	1 day	Patient expired still born baby, 3½ lb
31	A N and M	1 1	27	1 0	10,900		+	+	+	2½	+	+	+	+	General and induction of abortion	Few days	Slightly improved
32	A N	1 2	18	0 75	3,000		+	+	+	½	+	+	+	—	Liver and iron	90 days	Improved premature, mother and child alive
33*A	M.	1 5	30	1 0	3,000	4 3	+	+	+	½	+	+	+	+	Marmite	15 days	
															Liver	20 days	Improved
*B	P M.	1 0	20	1 0	4,000	4 3	+	+	+	2	+	+	+	+	Liver	30 days	Improved
															Marmite	21 days	Stationary

* 33 A and B are extra series

N B—All the blood counts given above were taken at the time of admission no end results are given above

Key—A N = Ante natal M = Maternity P M = Post maternity

Protocol XI

The morbidity and mortality

Series		Full term	Premature					Total
			5th month	6th month	7th month	8th month	9th month	
Hospital for Women and Children, Madras, 1931 (32 cases)	Cases	9	2	2	4	10	5	23
	Per cent	28 2	71 8					

Infants born and discharged alive	13	40 6 per cent
Still born	16	50 9 „
Died soon after delivery	3	9 4 „
Maternal mortality (Puerperal sepsis 1, Heart failure 5, Shock after section 1)	7	22 0 „
Condition very bad before discharge	4	12 5 „
Mothers discharged alive with condition improving	21	65 5 „
Puerperal sepsis complicating	4	12 5 „

Protocol XII

Hæmoglobin percentage, red cell count, and colour index on the average, of newly-born infants

Series	Number of cases	Hb per cent	R B C in millions	Colour index
Controls	15	110	5 3	1 03
Anæmic cases	10	105	5 1	1 028

We wish to express our thanks to the Superintendent, Government Hospital for Women and Children, and to Dr E A Menon, F R C S (E) for the facilities afforded, to the Director, King Institute and the Professor of Biochemistry, Medical College, for kindly helping in the bacteriological and biochemical studies

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AN INVESTIGATION INTO THE CAUSATION OF ASCITES

BY

M V RADHAKRISHNA RAO, MB, BS,
Research Fellow, Andhra University
(From the Department of Pathology, Medical College, Vizagapatam)

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WHILE proceeding with an investigation into 'Decompensated Portal Cirrhosis of the Liver' the problem which confronted me was the differential diagnosis of ascites In the majority of cases of ascites it was possible to diagnose the cause from the routine physical and clinical examination alone, but there is a small group of cases in which the clinical examination does not disclose any of the definitely known causes of ascites and the causation remains obscure even after thorough investigation The opportunities afforded to me in the wards of the King George Hospital, Vizagapatam, where many cases of ascites are treated every year, made the investigation into the causation of ascites in this part of the country possible

From time to time reports have been published in the medical literature in India on ascites of obscure origin Megaw (1921, 1924) was the first to point out 'chronic dysenteric peritonitis' as a probable cause of a common form of ascites in the tropics There has been considerable difference of opinion about the rôle of dysentery in the production of ascites According to Megaw, ascites following an attack of bacillary dysentery is due to chronic peritonitis, set up by the passage of irritant toxins of the dysentery bacilli from the lumen of the bowel to the peritoneum That ascites in these cases was due to previous dysenteric infection was concluded from the history of dysentery prior to the onset of ascites and from the positive Widal's agglutination tests against the bacilli of Shiga and Flexner Bose and Banerji (1929), investigating some of the obscure cases of ascites in Patna, came to the conclusion that the causation was probably 'due to disturbance of the absorptive power of the upper part of the peritoneum caused by previous dysenteric infection' Nigam (1927) described several cases of ascites in

Lucknow resulting from a slow fibrosis of the peritoneum due to an unknown toxin and called it 'endemic ascites' Though he is of opinion that a toxin is responsible for the fibrosis of the peritoneum, he disagrees with Megaw's theory that the dysenteric toxins are the causative factors Rogers (1913) was the first to draw attention to the fact that in the cases in which ascites followed dysentery, the causative lesion was cirrhosis of the liver Cunningham (1923), discussing the complications of bacillary dysentery, considered that in cases of ascites with a history of previous dysentery the liver was the primary cause of the affection as it showed advanced fibrotic changes In a paper recently submitted to the last conference of the All-India Medical Association at Calcutta, on 'the rôle of dysentery in the causation of ascites, from a study of post-mortem records and observations', Tirumurti and Radhakrishna Rao (1932) conclude as follows —

(*i*) 'Post-mortem statistics collected at the Medical College Hospital (King George Hospital), Vizagapatam, show that most of the cases of ascites are due to decompensation of the heart or cirrhosis of the liver

(*ii*) Though bacillary dysentery is common in these parts, no case of ascites due to chronic dysenteric peritonitis (Megaw) was seen post-mortem

(*iii*) In cases in which thickening of the peritoneum was associated with ascites, the causation of ascites was due to factors other than chronic dysenteric peritonitis

(*iv*) Thickening of the peritoneum, if present, need not always be the cause of ascites but may be its result

(*v*) We are of opinion that, in cases in which ascites may develop as a sequel to chronic dysentery, the causative factor is cirrhosis of the liver and not fibrosis of the peritoneum'

In this paper, I wish to present my observations on the causation of ascites in this district, and to discuss the rôle of dysentery in its causation and the value of Widal's agglutination tests against the bacilli of Shiga and Flexner, on the data collected by clinical investigation of cases under my observation

Investigation

A detailed investigation has been carried out on cases of ascites admitted into the Medical Wards of the King George Hospital, with a view to determine the cause of ascites In most of the patients, it was not possible to follow up their progress after discharge from the hospital On many of those who died in the hospital an autopsy was made

From July 1931 to the end of February 1932, 69 patients suffering from ascites were examined and the results obtained on the investigation carried out are tabulated in Table I

In 47 cases, the cause of ascites was determined from the physical and clinical examination alone, the results of further investigation only confirming the diagnosis

TABLE I

Showing the cause of ascites in 69 cases investigated

Serial number	Cause of ascites	Cases in which the diagnosis was definite (47 cases)	Cases in which the diagnosis was arrived after complete investigation (22 cases)	TOTAL	Percentage
1	Decompensation of the heart	15	1	16	23 3
2	Kidney inefficiency	10		10	14 5
3	Ascites associated with malignant tumours of the abdomen	2		2	2 9
4	Tubercular peritonitis	4		4	5 8
5	*Cirrhosis of the liver* — (*a*) Portal cirrhosis	11	10	21	30 4
	(*b*) Syphilitic cirrhosis	1	7	8	11 6
	(*c*) Infantile biliary cirrhosis	1		1	1 4
	(*d*) Associated with Banti's disease	2		2	2 9
6	Ascites associated with sub acute yellow atrophy of the liver	1		1	1 4
7	Extreme anæmia		3	3	4 4
8	Chronic peritonitis		1	1	1 4

In 15 cases in the above series, the ascites was due to failure of compensation of the heart as evidenced by marked dyspnœa, œdema of the legs, pulsating veins at the root of the neck, cyanosis and enlargement of the liver In 8 cases, paracentesis abdominis was done to relieve the dyspnœa and the fluid did not accumulate again after rest and treatment Only in one case had paracentesis to be repeated None of these patients showed any signs of fluid in the abdomen at the time of discharge from the hospital In most of them the diagnosis was further confirmed by the electro-cardiograph, and by the roentgenogram of the chest There was one death in this series, but a post-mortem examination in that case was not permitted

In 10 cases, the ascites was only a localized manifestation of the general anasarca due to kidney inefficiency The urine, which was very scanty, contained much protein and many casts Biochemical examination showed retention of nitrogenous bodies in the blood The efficiency of the kidney as estimated by MacLean's

(1927) urea concentration test was definitely deranged Paracentesis abdominis was performed in 4 cases as the ascites was causing pressure symptoms, and only in one case had it to be repeated These patients improved under treatment and there was no ascites at the time of their discharge from the hospital The post-mortem findings in one patient in this series, who died in the wards showed that secondary contracted kidneys was the cause of kidney inefficiency and ascites

In 2 cases, the ascites was associated with malignant tumours of the abdomen, in one, it was a malignant growth of the head of the pancreas with complete obstructive jaundice in an old man, and in the other, it was a malignant growth of the right ovary in a middle-aged woman

All the 4 cases of ascites due to tubercular peritonitis showed definite signs of tuberculosis of the lungs, which was confirmed by the skiagram of the chest Von Pirquet's test was positive in all the cases In 2 patients who died in the hospital, the ascites was due to acute miliary tuberculosis of the peritoneum, confirmed by post-mortem examination The other two patients improved gradually by general hygienic treatment and exposure of the abdomen to ultra-violet rays

In 15 patients the ascites was due to cirrhosis of the liver In one patient, a child aged 2 years, the ascites was due to syphilitic cirrhosis of the liver, the abdominal veins were prominent there was a strong positive Wassermann reaction of the blood and ascitic fluid, the liver was firm and enlarged to 3 inches below the costal margin Infantile biliary cirrhosis of liver was the cause of ascites in a child aged one and a half years, and this diagnosis was verified post-mortem In 2 cases, diagnosed as portal cirrhosis, it was found on post-mortem examination that the cirrhosis of the liver was a part of the syndrome described by Banti Histologically, the liver showed multilobular cirrhosis and the appearances of the spleen were typical of Banti's disease Eleven cases showed all the signs of portal obstruction with very prominent veins in the abdominal wall, ascites and enlargement of the spleen There was deranged function of the liver in all these patients in whom urobilin or urobilinogen was present in the urine The general condition was very poor with the 'hepatic facies', emaciated extremities and protuberant abdomen Paracentesis of the abdomen was done in these cases to relieve the pressure symptoms, and the patients were discharged after a varying period of stay in the hospital with no improvement in their condition The diagnosis was verified post-mortem in 2 cases who died in the hospital, and the appearances of the liver, macroscopic and microscopic, were those of Laennec's cirrhosis

In one case, it was found on post-mortem examination that the ascites was associated with sub-acute yellow atrophy of the liver There was diffuse destruction of the parenchyma of the liver, and no other definite pathological lesion was found to account for the ascites This case will be again referred to under discussion

In all the above cases the possibility of 'chronic dysenteric peritonitis' being the cause of ascites was kept in mind The results of the agglutination tests with the dysentery organisms in most of these cases are given in Tables VIII to XII, for control purposes

In the above series of 47 cases, 9 were examined post-mortem

The results of investigation in the 22 cases, in which the cause of ascites was not definite at the outset, are given in the following tables for discussion An explanation of the tests employed in this investigation is given below

Liver efficiency tests

In spite of the recent advances in biochemical methods, it has not been possible to estimate correctly the functional capacity of such an important vital organ as the liver It must be noted that the liver has a great reserve power and its ability to regenerate is remarkable, especially in chronic liver cell damage, as proved by the experiments of Mann and Bollman (1926) These factors must be taken into consideration while interpreting the results of the functional tests While a positive test (marked as + or ++ in Table II) is definitely in favour of hepatic dysfunction, a negative test (marked as +++ in Table II) does not exclude hepatic disease, but only indicates that there is enough functionally active liver tissue to carry on its functions

(*a*) *The lævulose tolerance test* of Strauss (1901) and Shirokauer (1913), slightly modified, was applied to determine the function of the liver The test is preceded by a 12-hour fast and the patient takes no breakfast, a sample of blood is drawn from the vein and lævulose dissolved in water is given by mouth, according to the weight of the patient, as recommended by Spence and Brett (1921) Four specimens of blood are then collected at half-hourly intervals and the blood-sugar is estimated by the method of Folin and Wu (1920) It has been shown by King (1927), Elmer and Scheps (1930), that in normal persons, the highest rise of blood-sugar above the fasting level was never more than 20 mg per 100 c c Tallerman (1923) found that a blood-sugar exceeding 0 135 per cent, or showing a rise of 30 mg above the initial fasting level, indicated a definite derangement of the hepatic function These standards are adopted in classifying the function of the liver A rise of blood-sugar above 30 mg is taken as definite impairment and a rise above 20 mg, but below 30 mg, as slight impairment of the liver function The liver function is considered good, if the rise of blood-sugar is below 20 mg

The results of the lævulose tolerance test are given in Table II The liver function was good in 5 cases, slightly impaired in 4, and definitely impaired in 8 cases

TABLE II

Liver efficiency tests

Serial number	Case number	LÆVULOSE TOLERANCE TEST (BLOOD SUGAR IN MG PER 100 C C)						Liver efficiency	WIDAL'S HÆMOCLASIC CRISIS				Liver efficiency	VAN DEN BERGH'S REACTION			
														DIRECT			INDIRECT
		Before lævulose	Half an hour after	One hour after	One and a half hours after	Two hours after	Rise of blood sugar		Before giving milk	Twenty minutes after	Forty minutes after	Rise or fall of W B C count		Immediate	Biphasic	Delayed	
1	1	36 2	105 3	95 2	95 2	86 9	19 1	+++	2,812	1,875	1 563	— 1,249	+	—	—	Strong	Positive
2	2	78 4	96 6	119 0	126 6	119 7	48 2	+	3,000	3,100	3,200	+ 100	+	—	—	Faint	,,
3	7	89 7	107 5	140 8	150 4	139 9	60 7	+	5,320	4,800	4 650	— 670	+	—	—	Strong	,,
4	8	82 0	76 6	83 0	99 0	101 5	18 5	+++	5,650	5,250	4,800	— 850	+	—	—	,,	,,
5	12								5,350	4,260	4,185	— 1,165	+	—	—		
6	19	88 9	85 1	103 6	89 7	90 9	14 7	+++	8,438	9,688	6 875	+ 1 250	++	—	—	,	,
7	20								4,500	5,200	5,500	+ 1,000	+	—	—	,	,
8	24	70 2	133 3	125 0	101 0	82 6	63 1	+	3,438	2,188	4,062	+ 624	+	—	+	—	,,
9	26	68 9	79 2	102 4	116 5	106 4	47 6	+	5,398	6,875	7,500	+ 2 102	++	+ (8 065 units)	—	—	,
10	34								10,530	16,250	12 500	+ 5 720	+++	—	—	Strong	,,
11	42	99 0							2,688	4,062	2,688	+ 1,374	++	—	—	Faint	
12	46	69 2	64 5	67 1	66 6	66 6	No rise	+++	5 312	8 125	8,750	+ 3,438	+++	—	—	—	—
13	47	79 7	110 5	121 2	113 6	101 5	41 5	+	5,000	5,812	5,200	+ 812	+	—	—	—	—
14	51	78 5	96 4	97 5	116 5	99 6	38 0	+	6,150	7,200	7,240	+ 1,090	+	—	—	Faint	Positive
15	52	126 6	153 8	156 2	117 6	125 0	29 6	+	7,500	8,200	8,150	+ 700	+	—	—	—	Faint
16	53	89 7	101 0	108 7	92 2	102 6	19 0	+++	4,688	8,125	8,125	+ 3 437	+++	—	—	Faint	,,
17	80	94 3	109 9	116 3	107 5		22 0	++	8,438	7,031	9,062	+ 624	+	—	—	—	—
18	57								13,125	14,375	18,750	+ 5 625	+++	—	—	Faint	Faint
19	62	78 7	81 3	81 4	102 6	94 3	23 9	++	2,500	5,625	3,438	+ 3,125	+++	—	—	—	Positive
20	72	73 3	79 4	84 0	74 1	114 9	41 6	+	8,388	9,375	7,500	— 888	+	—	—	—	—
21	79	88 9	97 1	102 6	111 7	107 5	22 8	++	1,720	2,185	2,969	+ 1,249	++	—	—	Faint	Faint
22	83	111 7	111 1	135 1	119 0	118 3	23 4	++	6 150	10,625	7,182	+ 1,475	+++	—	—	Strong	Positive

Liver efficiency — +++ = Good ++ = Slightly impaired + = Definitely impaired

(*b*) *The hæmoclasic crisis*, described by Widal, Abrami and Iancovesco (1920), is believed to be of the nature of an anaphylactic blood reaction due to the passage of partially digested proteins from the gastro-intestinal tract into the systemic circulation, when the liver is diseased In such cases, the normal post-prandial leucocytosis is replaced by a leucopenia or by the absence of any rise in the count, accompanied by a fall in blood pressure This test has been verified by Wilson (1922), Bose and Banerji (1929), who found it to be a reliable test for the functional integrity of the liver A white cell count is taken on an empty stomach before breakfast, 7 ounces of milk are given to drink and the counts are taken at the end of 20 and 40 minutes If the rise in the white cell count at the end of twenty or forty minutes is more than 3,000, the function of the liver is considered to be good, if the rise is above 2,000 and below 3,000, the liver function is slightly impaired, and if the rise is below 2,000 or there is actual leucopenia, the liver function is definitely impaired

Table II shows that the liver function, as estimated by this reaction, was good in 6 cases slightly impaired in 4 and definitely impaired in 12 cases

(*c*) *The pigmentary function* of the liver was estimated by the chemical method described by van den Bergh (1918) The improved technique as described by McNee and Keefer (1925) was adopted in this investigation The qualitative reaction was done in all the cases and the quantitative only in those cases with marked jaundice

By itself, this test may not be of much value, but when considered along with the other tests it is useful in determining the functional capacity of the liver

In 4 cases, both the direct and the indirect reactions were negative in 2, only the indirect was positive, 1 case showed direct immediate and 1 biphasic, while all the others showed delayed positive reaction

(*d*) In all the cases, the urine was examined for urobilinogen by Ehrlich's (1886) aldehyde reaction and for urobilin, by Schlesinger's (1903) test

Kidney efficiency tests

(*a*) Samples of blood for biochemical analysis were collected in the morning on a fasting stomach, coagulation being prevented by the use of a small quantity of finely powdered lithium oxalate Blood urea was estimated by MacLean and De Wesselow's (1927*a*) modification of Marshall (1913), van Slyke and Cullen's (1914) method Non-protein nitrogen and creatinin were estimated by the method of Folin (1923, 1923*a*) The method of Myers and Wardell (1918) was employed in the determination of the plasma cholesterol

The results of the blood analysis are given in Table III The normal standards given by Harrison (1930) are taken for comparing with these readings

TABLE III

Kidney efficiency tests

Serial number	Case number	Biochemical examination of blood (mg per 100 c c)				Urea concentration test (Maclean)				Total chlorides in urine (Percentage)	Albumin in urine (Qualitative)	Casts in urine	Kidney efficiency	Remarks
						One hour after		Two hours after						
		Urea	Non-protein nitrogen	Creatinin	Cholesterol	Volume of urine passed in c c	Percentage of urea	Volume of urine passed in c c	Percentage of urea					
1	1	26 2	20 64	1 86	142 0	40	0 4695	135	1 5050	1 334	No	No	++	
2	2	36 0	30 56	2 68	168 2	86	2 432	68	2 684		A trace	No	+++	
3	7	26 0	20 65	2 64	168 62	65	1 965	88	2 265		No	No	+++	
4	8					120	1 892	105	2 024	0 892	No	No	+++	
5	12	24 2	20 65	1 65		65	1 062	82	1 812	0 972	A trace	No	++	Albumin disappeared after tapping the abdomen.
6	19	9 0	33 7	1 695		60	2 359	28	2 472	2 03	No	Hyaline and granular	++	Urobilin and urobilinogen present in urine
7	20					36	3 859	15	3 836	0 116	No	No	+++	
8	24	15 5	26 67	2 25	109 33	40	2 297	57	1 515	1 0875	No	No	+++	
9	26	85 6	55 56	1 66		22	3 094	20	3 251	1 003	No	No	++	
10	34		37 26	1 57		32	4 668	20	5 054	0 3779	A trace	No	+++	
11	42	15 0	25 2		45 45	46	1 749	36	2 064	0 8	A trace	Granular	++	
12	46	78 0	57 61	2 4	76 2	150	0 7144	48	0 7905	0 7605	No	No	+	
13	47	24 5	26 07		84 74	31	2 02	31	3 052	0 5733	No	No	+++	
14	51	55 0	40 53		193 2	7	2 461	18	3 181		No	No	+++	
15	52	28 0	30 0		101 6	35	3 602	36	3 588		No	No	+++	
16	53	11 0	23 07		42 5	192	0 6002	123	0 9319	0 8816	No	No	+	Urobilinogen present in urine
17	80	36 5	17 55			108	2 012	96	2 656		No	No	+++	
18	57					26	3 719	83	3 229	1 053	No	No	+++	
19	62	39 0	30 5	1 65	88 26	30	1 5769	35	1 8480		No	Hyaline and granular	++	Urobilinogen present in urine
20	72	28 0	15 0	2 15	128 25	125	1 461	86	2 295		A trace	No	+++	
21	79	54 5	32 61	2 564	76 3	26	2 045	25	2 636		No	No	+++	Urobilin present in urine
22	83	26 5	13 39	2 212		88	1 2012	45	1 4476		No	No	++	Urobilin present in urine

Kidney efficiency — +++ = Good ++ = Fair + = Bad

(*b*) The urea concentration test was done according to the technique given by MacLean (1927), in children, the dose of urea given by mouth was regulated according to the age, as recommended by Harrison (1930*a*) The urea was estimated by the hypobromite method 'If either specimen gives a percentage of urea above 2, the kidneys are held to be fairly efficient, the higher the concentration, the more effective is the renal function' (MacLean, 1927)

(*c*) The chlorides in urine were estimated by the Whitehorn's (1921) method All the patients were on the hospital 'Ordinary-Indian' diet, and a twenty-four hour sample of urine was taken for the estimation

(*d*) Routine physical, chemical and microscopic examination of the urine was done in all the cases

Table III shows that the kidney efficiency was good in 13 cases, fair in 7 and bad in 2 cases

Examination of the heart

There was no organic disease of the heart in any of these 22 cases In 3 patients, Nos 46, 53 and 83, there were hæmic murmurs, and extreme anæmia In one patient, No 80, with a soft systolic murmur in all the areas, the myocardium was badly damaged and the radiograph showed a dilated heart In all the other patients the condition of the heart was good, except for the cardiac embarrassment caused by the pressure of the fluid in the abdomen

Examination of the blood

Table IV shows that 14 patients in this series had leucopenia, while 2 showed leucocytosis, marked anæmia was present in 12, 1 patient, No 47, whose blood showed marked eosinophilia had a heavy ankylostome infection

The blood coagulation time was determined by Ricker's coagulometer (Brodie-Russel-Boggs pattern) The normal coagulation time of the blood, as determined by this instrument, is about 5 minutes Uniformly accurate results were given by this instrument in all the normal controls The coagulation time was normal in 11 and delayed in 11 patients

Wassermann reaction of the blood was strongly positive in 10 patients, moderately positive in 4 and weakly positive in 3, in 5 patients the reaction was negative

The fragility of the red blood cells was determined by the method described by Panton and Marriack (1927) The fragility was normal in 7, and diminished in 15 cases The increased resistance to hæmolysis is seen in cases of obstructive jaundice and splenic anæmia (Beaumont and Dodds, 1931) Cases of extreme anæmia, with a direct delayed van den Bergh's reaction, may also show diminished fragility of the red blood cells Hughes and Shrivastava (1927) have shown that in advanced

TABLE V

Examination of ascitic fluid

Serial number	Case number	Specific gravity	Colour	Clear or turbid	Quantity of albumin (in gram per cent)	Spontaneous coagulation	Coagulation on standing	Number of cells (per c.mm.)	Wassermann reaction	Culture	Transudate or exudate
1	1	1,012	Straw	Clear	0·65	No	No	473	Strongly positive	Negative	Transudate
2	2	1,005	,,	,,	0·32	No	No	260	Negative	Gram negative motile bacillus not conforming to any biochemical tests for known pathogens grown	,,
3	7	1,008	,,	,,	0·86	No	No			Negative	,,
4	8	1,005	,,	Slightly turbid	0·90	No	No	380		Gram negative diplococci grown	,,
5	12	1,010	,,	,,	1·44	No	No	180	Negative	Negative	,,
6	19	1,010	,,	Opalescent	0·30	No	No	776	Strongly positive	,,	,,
7	20	1,005	,,	Clear	0·09	No	No	478	,,	Staphylococci grown	,,
8	24	1,005	,,	,,	0·06	No	No	80	,,	,,	,,
9	26	1,006	,,	,,	0·80	No	No	60	Weakly positive	Negative	,,
10	34	1,004	Light straw	,,	0·64	No	No	22	Strongly positive		,,
11	42	1,006	Straw	,,	0·24	No	No	288	Negative	,,	,,
12	46	1,007	,,	,,	1·90	No	No	180	Strongly positive	,,	,,
13	47	1,010	,,	Slightly turbid	2·00	No	No	65	Moderately positive	,,	,,
14	51	1,005	Pale straw	,,	0·15	No	No	187	Strongly positive	,,	,,
15	52				(Paracentesis of the abdomen not done)						
16	53	1,007	Pale straw	Slightly turbid	0·30	No	No	148	Weakly positive		,,
17	80	1,008	Pale whitish	Opalescent	0·45	No	No	262	Negative		,,
18	57	1,007	Straw	Clear	0·75	No	No	40	Strongly positive		
19	62	1,008	Light straw	,,	0·10	No	No	58	,,		
20	72	1·004	Pale whitish	Opalescent	0·05	No	No	28	,,		
21	79				(Paracentesis of the abdomen not done)						
22	83				(Paracentesis of the abdomen not done)						

Table VI

Short notes of the cases

Serial number	Case number	Age (in years)	Sex	Enlargement of liver (in inches below costal margin)	Enlargement of spleen (in inches below costal margin)	Veins of the anterior abdominal wall	Hepatic facies	General condition	Number of times tapped in hospital	Result	Remarks
1	1	16	Male	4 inches	6 inches	Faintly seen	No	Poor	5	Relieved	No ascites when the patient was seen 4 months after he was discharged from the hospital
2	2	45	,,	No	3 ,,	Prominent	No	Fair	6	,,	
3	7	60	,,	No	3 ,,	Faintly seen	No	,,	1	,,	
4	8	40	,,	No	4 ,,	Very prominent	No	Poor	8	No improvement	Omentopexy done—No improvement (Portal cirrhosis verified at operation)
5	12	22	,,	No	No	Not marked	Present	Very poor	2	Died	A post mortem could not be done
6	19	35	,,	No	No	Present	No	Fair	4	No improvement	Guinea pig inoculated with ascitic fluid, died
7	20	4	Male child	2 inches	3 inches	Very marked	No	,,	4	,,	Surface of the liver is hard and granular on palpation
8	24	28	Male	No	4 ,,	Present	Present	Poor	3	Relieved	
9	26	30	,,	No	No	Not marked	,,	Very poor	1	No improvement	Deep jaundice present
10	34	5	Female child	2 inches (left lobe)	3 inches	Very marked	No	Good	2	,,	Surface of the liver is hard and granular on palpation
11	42	16	Male	2 inches (left lobe)	5 ,,	Present	No	Fair	4	Died	Autopsy—Portal cirrhosis of the liver
12	46	50	,,	No	No	,,	No	,,	1	Relieved	
13	47	35	,,	No	No	Not present	No	,,	1	,,	
14	51	50	,,	No	No	Present	Present	Very poor	1	No improvement	
15	52	35	,,	No	4 inches	Not present	No	Fair	*Nil*	Relieved	
16	53	50	,,	No	1 inch	,,	No	,,	1	,,	
17	80	35	Female	No	No	,,	No	,,	1	,,	
18	57	13	Male	2 inches (left lobe)	½ inch	Present	Present	Poor	6	Still in hospital	Surface of the liver is hard and granular on palpation
19	62	35	,,	No	No	Faintly seen	,,	Very poor	9	No improvement	
20	72	30	,,	No	No	Few present	,,	Poor	3	,,	
21	79	35	,,	No	4 inches	Present	,,	Very poor	*Nil*	,,	
22	83	40	,,	No	4 ,,	Not marked	No	,,	*Nil*	Relieved	

Short notes of the cases

It is not possible in this short paper to give a detailed account of each case short notes of each of the cases are given in Table VI

Enlargement of the liver was present only in 2 cases (Nos 1 and 20) In cases Nos 20 31 12 and 57 the left lobe of the liver was hard and granular when palpated after paracentesis of the abdomen Enlargement of the spleen was present in 12 cases

There were two deaths in this series, in one (No 12) post-mortem examination was not allowed but in the other (No 12) autopsy showed a multilobular cirrhosis of the liver, in this latter case there were dense adhesions between the diaphragm and the spleen, the capsule of which was much thickened Histologically the appearances of the liver were those of multilobular cirrhosis and those of the spleen, chronic passive venous congestion

The patient in case No 1 showed marked improvement during his stay of four and a half months in the hospital A course of anti-syphilitic treatment was given, as the blood and ascitic fluid Wassermann reactions were positive On the two occasions on which he reported himself after discharge from the hospital there was no fluid in the abdomen and the enlargements of the spleen and liver were considerably diminished

Omentopexy was done in case No 8, but it proved to be a failure, as the abdomen filled up rapidly necessitating repeated paracentesis A coarsely nodular cirrhosis of the liver was found at operation

Widal's agglutination tests with the dysentery organisms

Widal's test against the bacilli of Shiga and Flexner was carried out in the present series of cases, as well as in a number of controls

The standard agglutination method of Dreyer (described by Stitt, 1923) was employed in carrying out the tests Most of the workers on bacillary dysentery, e g, Manson-Bahr (1922), Rogers (1930), Perry (1925), Acton and Knowles (1928) and Megaw (1924), agree that the macroscopic method is by far the best In the case of the Flexner group, a mixture of the various strains of the organisms was used for the agglutination test The test is considered positive by Manson-Bahr (1922) and Rogers (1929), when the agglutination occurs in a dilution of 1 in 25 in the case of *B dysenteriæ* (Shiga) and 1 in 50, in *B dysenteriæ* (Flexner) These standards were adopted in classifying the results of the agglutination tests Both the blood serum and the ascitic fluid were tested for their agglutination reaction Dilutions of the sera and the ascitic fluid were made with sterile saline in multiples of 16, as recommended by Stitt (1923*a*)

It will be seen from Table VII, that out of the 22 patients in this series, there was a history of dysentery in 6, though only in 4 the dysentery occurred before the

onset of ascites The agglutination tests, either of the blood or ascitic fluid or both, were positive in 13 and negative in 9 cases

TABLE VII

Agglutination tests with dysentery organisms (Present series of 22 cases)

Serial number	Case number	Duration of ascites (in months)	History of dysentery (Period in months from the date of attack)	AGGLUTINATION TESTS			
				BLOOD		ASCITIC FLUID	
				B dysenteriæ (Shiga)	*B dysenteriæ* (Flexner)	*B dysenteriæ* (Shiga)	*B dysenteriæ* (Flexner)
1	1	8	No	Negative	Negative	+ 1/32	+ 1/32
2	2	3	No	,,	,	Negative	Negative
3	7	2	No	,,	,,	,,	,
4	8	5	No	,,	,,	,,	,
5	12	8	No	+ 1/128	+ 1/128	,,	+ 1/32
6	19	1½	No	+ 1/16	+ 1/32	,,	Negative
7	20	2	No	Negative	+ 1/128	,,	,,
8	24	48	No	,,	Negative	,,	,,
9	26	2	No	+ 1/128	+ 1/128	+ 1/32	+ 1/32
10	34	1	No	+ 1/128	+ 1/128	Negative	Negative
11	42	5	No	Negative	+ 1/16	,,	,,
12	46	1½	½	,,	+ 1/128	,,	+ 1/128
13	47	2	3	,,	Negative	,,	Negative
14	51	2	1	,,	+ 1/128	,,	,,
15	52	5	No	,,	+ 1/128		
16	53	1	No	,,	+ 1/128	Negative	Negative
17	80	1	4	,,	+ 1/128	,,	+ 1/64
18	57	4	No	,,	Negative	,,	+ 1/32
19	62	4	24	+ 1/32	+ 1/128	,	Negative
20	72	2	No	Negative	+ 1/128	,	,,
21	79	2	No	,,	+ 1/128		
22	83	3	12	+ 1/32	Negative		

A number of control cases with and without ascites, were tested for their agglutination reactions against the bacilli of Shiga and Flexner, to determine the value of these tests in the diagnosis of the cause of ascites

TABLE VIII

Agglutination tests with dysentery organisms in typical cases of ascites due to cirrhosis of the liver

Serial number	Case number	Duration of ascites (in months)	History of dysentery (Period in months from the date of attack)	AGGLUTINATION TESTS				Disease
				Blood		Ascitic fluid		
				B dysenteriæ (Shiga)	*B dysenteriæ* (Flexner)	*B dysenteriæ* (Shiga)	*B dysenteriæ* (Flexner)	
1	3	12	No	Negative	+ 1/128			Portal cirrhosis
2	18	3	1		+ 1/32			,,
3	21	3	(20 years)	+ 1/128	+ 1/128	Negative	Negative	,,
4	33	12	6			,,	,,	Banti's disease—early cirrhosis
5	11	2½	6	+ 1/128	+ 1/64	+ 1/16	+ 1/32	Portal cirrhosis
6	55	12	No	+ 1/32	+ 1/128	Negative	Negative	,,
7	58	3	No			,,	+ 1/128	Syphilitic cirrhosis
8	67	3	No	Negative	+ 1/128	,,	+ 1/32	Early portal cirrhosis
9	78	2	3	,,	Negative			Portal cirrhosis

TABLE IX

Agglutination tests with dysentery organisms in typical cases of ascites due to kidney inefficiency

Serial number / Case number	Duration of ascites (in months)	History of dysentery (Period in months from the date of attack)	AGGLUTINATION TESTS				Disease
			Blood		Ascitic fluid		
			B dysenteriæ (Shiga)	*B dysenteriæ* (Flexner)	*B dysenteriæ* (Shiga)	*B dysenteriæ* (Flexner)	
17	5	No	Negative	Negative	Negative	Negative	Diffuse glomerular nephritis
3	5	No	+ 1/32	+ 1/128	+ 1/32	+ 1/32	Chronic nephritis
5	18	No	Negative	+ 1/128	Negative	+ 1/128	Chronic ,, parenchymatous nephritis
8		No	,,	Negative	,,	Negative	
		No	,,	+ 1/128			Sub acute nephritis

TABLE X

Agglutination tests with dysentery organisms in typical cases of ascites due to decompensation of the heart

Serial number	Case number	Duration of ascites (in months)	History of dysentery (Period in months from the date of attack)	AGGLUTINATION TESTS				Disease
				Blood		Ascitic fluid		
				B dysenteriæ (Shiga)	*B dysenteriæ* (Flexner)	*B dysenteriæ* (Shiga)	*B dysenteriæ* (Flexner)	
1	14	2	No	Negative	Negative	Negative	Negative	Endocarditis, mitral valve
2	16	6	No	,,	+ 1/16	,,	+ 1/16	Mitral regurgitation
3	23	1	No	,,	+ 1/16	,,	Negative	Syphilitic myocarditis
4	31	4	No	,,	+ 1/16	,,	,,	Mitral regurgitation
5	32	6	No	+ 1/64	+ 1/16	,,	,,	Double mitral
6	40	12	No	Negative	+ 1/128	,,	+ 1/32	Adhesive pericarditis
7	53	12	No	,,	+ 1/128	,,	+ 1/128	Double mitral
8	77	1	No	,,	Negative	,,	Negative	,,
9	68	7	8	,,	+ 1/128	,,	,,	,,
10	86	2	4	+ 1/64	Negative	,,	,,	Mitral regurgitation

TABLE XI

Agglutination tests with dysentery organisms in other cases of ascites

Serial number	Case number	Duration of ascites (in months)	History of dysentery (Period in months from the date of attack)	AGGLUTINATION TESTS				Disease
				Blood		Ascitic fluid		
				B dysenteriæ (Shiga)	*B dysenteriæ* (Flexner)	*B dysenteriæ* (Shiga)	*B dysenteriæ* (Flexner)	
1	11	4	No	Negative	Negative	Negative	Negative	Infantile biliary cirrhosis
2	22	6	No	,,	,,	,,	,,	Acute miliary tuberculosis (verified post mortem)
3	25	5	1	+ 1/64	+ 1/16			Acute yellow atrophy of liver (verified post mortem)
4	43	2	No	+ 1/128	Negative	+ 1/16	Negative	Acute miliary tuberculosis with Banti's disease (verified post-mortem)
5	85	2	No	Negative	,,	Negative	,,	Extreme anæmia (secondary to heavy round worm infection)

TABLE XII

Agglutination tests with dysentery organisms in unselected (control) cases without ascites

Serial number	Case number	History of dysentery (Period in months from the date of attack)	AGGLUTINATION TESTS		Disease
			Blood		
			B. dysenteriæ (Shiga)	*B. dysenteriæ* (Flexner)	
1	13	No	Negative	Negative	Syphilitic cirrhosis of the liver
2	38	6	+ 1/128	+ 1/128	Catarrhal jaundice
3	39	No	Negative	Negative	Syphilitic cirrhosis of the liver
4	44	No	,,	+ 1/128	Hypertrophic biliary cirrhosis
5	71	No	,,	Negative	Obstructive jaundice
6	M_2—E	No	,,	,,	Pernicious anæmia
7	P_2—6	½	,,	+ 1/128	Acute bacillary dysentery
8	82	12	+ 1/32	+ 1/128	Syphilitic cirrhosis of the liver.
9	84	No	+ 1/64	Negative	,,
10	89	No	+ 1/32	+ 1/128	Ankylostomiasis
11	90	No	Negative	+ 1/64	,,
12	91	2	+ 1/64	Negative	Peripheral neuritis
13	M_2—25	6	+ 1/32	,,	Myocarditis

Table VIII shows the results of the agglutination tests in typical cases of ascites due to cirrhosis of the liver, described in Table I In 3 cases, there was a history of dysentery before the onset of ascites and 6 cases showed positive agglutination reactions

Out of the 5 cases of ascites due to kidney inefficiency given in Table IX, only one gave a history of dysentery before the onset of ascites Agglutination tests were positive in 3 cases and negative in 2 cases

Out of the 10 cases of ascites due to decompensation of the heart, shown in Table X, 2 gave a history of dysentery before the onset of ascites and 5, positive agglutination tests

The results of the agglutination tests in other cases of ascites are given in Table XI A history of dysentery was given in 1 case and the agglutination tests were positive in 2 cases

Table XII shows the agglutination tests in unselected patients without ascites There was a history of dysentery in 5 of them, agglutination tests were positive in 8, while 5 gave a negative reaction

A summary of the results of the agglutination tests with dysentery organisms, in all the above cases, is given in Table XIII —

TABLE XIII

Summary of the results of the agglutination tests with dysentery organisms

Serial number	Table number	Total number of cases	Number of cases with history of dysentery	Number of cases without history of dysentery	Number of cases with a history of dysentery before the onset of ascites	Number of cases in which the agglutination tests were positive	Number of cases in which the agglutination tests were negative
1	Table VII	22	6	16	4	13	9
2	Table VIII	9	5	4	3	6	3
3	Table IX	5	1	4	1	3	2
4	Table X	10	2	8	2	5	5
5	Table XI	5	1	4	*Nil*	2	3
6	Table XII	13	5	8	*Nil*	8	5

DISCUSSION

(a) *Discussion of the cases* —In most of the above cases, the causative factor in the production of ascites can be determined by taking into consideration the results of the complete investigation

In 2 cases in this series under discussion, the diagnosis of portal cirrhosis was verified—in one case (No 8) at operation, and in the other (No 42) at autopsy

In 3 cases (Nos 46, 53 and 83), the ascites was associated with extreme anæmia due to ankylostomiasis These cases improved well under treatment Paracentesis of the abdomen was done in cases Nos 46 and 53, to relieve the pressure symptoms, and the fluid did not accumulate again In case No 83, there was no necessity for paracentesis on account of the small quantity of fluid present Liver function was good in all these cases and there was a history of dysentery prior to the onset of ascites, in one case only (No 83) Though the agglutination tests

were positive in all these cases a diagnosis of chronic peritonitis due to bacillary dysentery is not justified from these facts alone, as there was marked improvement under treatment and the fluid never re-accumulated after improvement in the general condition

A diagnosis of decompensation of the heart due to bad myocarditis, as the cause of ascites, was made in case No 80 which showed a dilated heart with systolic murmurs in all the areas and marked anæmia A history of repeated swellings of the body, with ascites off and on, was given in this case The positive agglutination tests in this case were due to an attack of dysentery before the last appearance of ascites The diminished function of the liver, in this case, may be due to its damage on account of the chronic passive congestion produced by repeated attacks of congestive heart failure

That syphilis was the cause of cirrhosis of the liver was concluded in cases Nos 20, 34 and 57, which showed a strongly positive Wassermann reaction, a hard and granular liver, enlarged spleen and prominent veins in the abdominal wall There was no history of dysentery in any of these cases, though cases Nos 20 and 34 gave positive agglutination tests The negative liver function tests, in cases Nos 34 and 57, only show that there was still sufficient amount of liver tissue which was functionally active The diagnosis of syphilitic cirrhosis of the liver in case No 1 was confirmed by his remarkable improvement with the disappearance of ascites, after a complete course of anti-syphilitic treatment The patient in case No 24 similarly improved after anti-syphilitic treatment It is presumed that, in these cases, the cirrhosis of the liver was not so far advanced as to cause permanent damage The patients in cases Nos 19 and 72, with diminished liver function and strongly positive Wassermann reaction of ascitic fluid, did not stay for a sufficiently long time in the hospital to complete a course of anti-syphilitic treatment for confirming the clinical diagnosis of syphilitic cirrhosis of the liver by the therapeutic test It must be mentioned, however, that the therapeutic test in these cases may not be always helpful in confirming the diagnosis, especially if the cirrhosis of the liver is far advanced There was no history of dysentery in these two patients, though in one (No 72), a positive agglutination reaction was present

In cases Nos 2, 7, 12, 26, 47, 51 52 and 79, the liver function was definitely impaired There was no history of dysentery prior to the onset of ascites in any of these cases except in No 47 The negative agglutination tests, after such a short period as 3 months after an attack of dysentery, are strongly suggestive that the dysentery in this case (No 47) was probably amœbic The clinical findings and the results of investigation are strongly in favour of portal cirrhosis being the cause of ascites in all these cases To what extent syphilis plays a part in the ætiology as judged by the positive Wassermann reactions does not seem to be very definite

In case No 62, the diagnosis is still a matter of conjecture, even after a complete investigation Marked anæmia, which was present, cannot be attributed as the cause of ascites, as the abdomen filled up very rapidly necessitating repeated paracentesis The clinical picture resembled that of a terminal stage of cirrhosis of the liver, but the results of the investigation did not confirm it At this stage, there should certainly be functional derangement of the liver, with enlarged spleen Though the 'aldehyde test' was positive in this case there was no other clinical or laboratory evidence to substantiate that kala-azar was responsible for the causation of ascites By a process of elimination, the ascites in this case had to be attributed to a chronic peritonitis There was a history of dysentery two years prior to the onset of ascites, which was of 4 months' duration The agglutination tests were positive for the bacilli of Shiga and Flexner How far the dysenteric infection is responsible for the chronic peritonitis could not be judged from the results of the clinical and laboratory investigations

Thus, in this series of 22 cases of ascites, the causative factor was portal cirrhosis in 10, syphilitic cirrhosis in 7, extreme anæmia in 3, decompensation of the heart in one and chronic peritonitis in another There was a history of dysentery before the onset of ascites in 4 and the agglutination tests were positive in 13 In all these patients except one (No 62), there were other definite evidences to account for the causation of ascites The relationship between chronic peritonitis and dysenteric infection in case No 62 could not be established from the data available

(*b*) *The association of ascites with kala-azar* —There is no evidence that the ascites in any of the above cases was due to that type of cirrhosis of the liver, which is occasionally seen in cases of kala-azar, as described by Rogers (1923*a*) The 'aldehyde test' was negative in all the patients but one (No 62) Even in this patient there was no enlargement of the spleen or liver and no pyrexia to suggest a diagnosis of kala-azar Kala-azar is not endemic in this district, and the few patients with this disease, who are sometimes seen in the wards, are imported from other endemic areas Thus, kala-azar does not play any part in the causation of ascites in this district

(*c*) *The value of the agglutination tests against the dysentery organisms in the diagnosis of the causation of ascites* —The results of the agglutination tests against the bacilli of Shiga and Flexner, tabulated in Table XIII, show that out of the 51 cases of ascites in which these tests were done, 29 (56 9 per cent) gave a positive reaction There was a history of dysentery in 15 cases (29 4 per cent) and in 10 (19 6 per cent) it occurred before the onset of ascites In all these cases except one (No 62), the ascites was found to be due to causes other than 'chronic dysenteric peritonitis' on investigation The positive agglutination tests in case No 62 only confirm the history that there was dysenteric infection, but does not throw any light on the question as to whether it was the cause of the chronic peritonitis

Out of the 13 cases without ascites, 5 (38 5 per cent) gave a history of dysentery and the agglutination tests were positive in 8 cases (61 6 per cent)

Thus out of the 61 cases there was a history of dysentery in 20 (31 3 per cent) and the agglutination tests were positive in 37 of them (58 8 per cent)

It may therefore be concluded, from the above data that positive agglutination tests [according to the standards given by Manson-Bahr (1922) and Rogers (1929)] are strongly in favour of a previous infection with the bacilli of dysentery The evidence is stronger if there is a history of dysentery as well These tests fail to provide any information as to how far this previous infection with the bacillary dysentery is responsible for the ascites in any case In the clinical investigation of ascites these positive agglutination tests help to the same extent as a positive Widal's reaction against the typhoid organisms in the diagnosis, they simply confirm a previous infection with the bacilli of dysentery or typhoid fever, as the case may be, these may or may not have any relation to the ascites

There is no evidence that the agglutinins occur in a higher proportion in cases of ascites than those found in non-ascites cases It may therefore be concluded that the percentage incidence of bacillary dysentery is the same in ascites and non-ascites cases Hence, a positive agglutination test or a history of dysentery in a case of ascites is of no special ætiological significance Moreover while a positive agglutination test is strongly in favour of a previous infection with dysentery organisms, a negative test does not exclude it, as the agglutinins rapidly disappear after the patient is cured

The fact that 58 8 per cent of all the cases showed positive agglutination tests denotes only that bacillary dysentery is very common in this district, the Flexner group of organisms seem to be the common invaders judging from the high proportion of agglutination tests with this group

The following case illustrates how the positive agglutination tests and a history of dysentery are of no value in arriving at a diagnosis as to the cause of 'ascites of obscure ætiology'

Case No 25 B Appala Naidu, a Hindu male, aged 35 years, was admitted into the medical wards of King George Hospital on 16th August, 1931, with a complaint of swelling of the abdomen and feet of 1 month's duration

Previous history indicated that he was subject to frequent attacks of malaria and he had an attack of dysentery about 5 weeks prior to his admission into the hospital Shortly after the dysentery, he noticed swelling of the abdomen and feet

On *physical examination*, he was found to be moderately nourished The visible mucous membranes were not anæmic and there were sub icteric tint of the scleræ and slight œdema of both the feet and legs, which pitted on pressure Fœtor of breath and pyorrhœa alveolaris were present There was marked distension of the abdomen with all the evidences of free fluid in it, no veins were visible on the abdominal wall, spleen and liver were not palpable

Respiratory and circulatory systems were normal, except for the pressure effects of the fluid in the abdomen Except for the lost knee jerks on both sides, there was nothing abnormal with the nervous system The temperature, pulse and respiration rates were normal

Laboratory findings Examination of the blood did not reveal anything abnormal, the blood counts, fragility of the red cells, examination of the blood smear were normal, Wassermann reaction was negative, van den Bergh's reaction was biphasic The agglutination tests were positive against the bacilli of Shiga and Flexner in dilutions of 1 in 64 and 1 in 16 respectively The examination of the urine was negative, except for the presence of urobilin

Fæces contained blood and mucus microscopically no amœbæ were found, but only red blood cells and plenty of degenerating leucocytes Culture of the fæces was negative for dysentery organisms

Diagnosis The positive agglutination tests, the history of dysentery prior to the onset of ascites and the other negative findings pointed to 'chronic dysenteric peritonitis' as being probably the cause of ascites in this patient

Progress The patient gradually became worse and died on 23rd September, 1931 Shortly before death there were petechial hæmorrhages over the chest and abdominal wall

Post mortem report Eight pints of straw coloured fluid were found in the abdominal cavity The visceral and parietal peritoneum was smooth and shiny The liver weighed only 24 ounces and was smaller in size than normal soft in consistency and yellowish brown in colour The lungs showed hypostatic congestion and the spleen passive congestion The large intestines showed a number of transverse ulcers along the free edges of the folds of the mucous membrane

Histologically, the appearances of the liver resembled those of sub acute yellow atrophy, with extensive necrosis of the liver cells and accumulation of pigment No amœbæ were found in the ulcers of the large intestine

Comment That the ascites was not due to 'chronic dysenteric peritonitis' was proved by the autopsy In the absence of other pathological evidence, the ascites has to be attributed to sub acute yellow atrophy of the liver The experimental researches of Bollman (1931) have shown that the liver is concerned in the metabolism of water and ascites is an event in failing hepatic function and is not necessarily dependent on portal obstruction

(*d*) *The rôle of dysentery in the causation of ascites*—The results of this investigation do not support the view of Megaw (1924), that 'the commonest form of ascites in India is not due to cirrhosis of the liver, but to a fibrosis of the peritoneum resulting from irritation of the peritoneum by the toxins of the bacilli of dysentery' The evidences which have been brought forward in support of this theory were, (1) history of an antecedent attack of dysentery shortly before the onset of ascites, (2) positive agglutination tests against the bacilli of Shiga and Flexner, and (3) in few cases, the post-mortem evidence of a diffuse fibrotic thickening of the peritoneum, especially in the upper part of the abdomen

That the onset of ascites was preceded by an attack of dysentery does not necessarily mean that the dysentery has in some way contributed to the development of ascites That a history of dysentery is of no particular value is evident from the results of this investigation where, in all the patients who gave a history of antecedent dysentery, the ascites was found to be due to causes other than 'chronic dysenteric peritonitis' When more than 50 per cent of the control cases show evidence of infection with the bacilli of dysentery, a history of dysentery, even if present, cannot be relied upon as establishing any ætiologic relationship in the development of ascites

It was already shown that, while positive agglutination tests against the bacilli of Shiga and Flexner are strongly in favour of previous infection with bacillary

dysentery they fail to prove that dysentery plays any part in the ætiology of ascites

In all the 5 cases described by Megaw in support of his view there was general fibrotic thickening of the peritoneum 3 cases showed ulceration of the intestines and 1 case was associated with cirrhosis of the liver Thickening of the peritoneum is found associated with a number of conditions and, unless all the other possible factors are excluded by a thorough examination, it should not be attributed to dysentery Osler and McCrae (1930) point out that 'in cases of *long continued ascites*, the serous surfaces generally become thickened and present an opaque, dead white colour This is observed especially in *hepatic cirrhosis*, but attends *tumours, chronic passive congestion* etc' Repeated paracentesis of the abdomen to relieve the pressure symptoms in ascites may result in chronic peritonitis due to a mild infection with micro-organisms of a low degree of virulence The commonest causes of simple chronic peritonitis stated by French (1928) are '*a former tuberculous peritonitis* from which the tubercles have disappeared, and the chronic inflammation which results from *repeated paracentesis abdominis* for any other variety of ascites *Granular nephritis* and *arterio-sclerosis* are considered by Hale-White (1888) as important factors in causing that form of chronic peritonitis of which a universal peri-hepatitis is a marked feature The results of post-mortem observations recorded in a previous paper (Tirumurthi and Radhakrishnarao, 1932) showed that in cases in which thickening of the peritoneum was associated with ascites, the causation of ascites was found to be due to factors other than 'chronic dysenteric peritonitis' Out of the 10 cases examined post-mortem in the pesent series of 69 cases of ascites investigated, chronic peritonitis was present only in 3 In 2 cases, it was associated with acute miliary tuberculosis of the peritoneum and in the other, with portal cirrhosis of the liver In the latter case, the peritoneum was opaque white in colour, peri-hepatitis and peri-splenitis were present and the macroscopic and microscopic appearances of the liver were typical of portal cirrhosis The intestines were normal in all these 3 cases

Thus, it is not easy to say whether the chronic peritonitis present in any case, is the cause or the result of ascites Even if evidences of bacillary dysentery, recent or old, are present in the intestines, the thickening of the peritoneum should not be attributed to it, unless all the other possible causes of such thickening are excluded

Moreover, none of the other investigators into bacillary dysentery, has mentioned 'chronic dysenteric peritonitis' leading to ascites as one of the complications of the disease 'The inflammatory process in bacillary dysentery is essentially confined to the mucous membrane, so that localized peritonitis is rare over the sites of the lesions' (Rogers, 1930) Hence, such a condition of 'chronic dysenteric peritonitis' must be rare and cannot therefore be the cause of a 'common

form of ascites in the tropics' The results of the clinical and pathological investigations into the causation of ascites in this district, where bacillary dysentery is very prevalent, does not support the view held by Megaw If the toxins of bacillary dysentery are responsible for ascites, more cases of this type should have been seen either clinically or at autopsy The post-mortem records show that even in fatal cases of bacillary dysentery, where the toxæmia would have been severe, there was no ascites and the peritoneum was not thickened and did not show any signs of inflammation

It may therefore be concluded from the above observations that 'chronic dysenteric peritonitis' is very rare and is not a common cause of ascites

Rogers (1913) and Cunningham (1923) have shown that hepatic cirrhosis with ascites may occur as a complication of bacillary dysentery Cunningham (1923) has pointed out that such a complication 'is of considerable importance on account of its relative frequency and its almost invariably fatal result' It is quite possible that the toxins of bacillary dysentery, especially in sub-acute and chronic cases, absorbed into the portal circulation may damage the liver cells and produce a type of cirrhosis which is closely allied to the 'sub-acute toxic cirrhosis' of Mallory The following case illustrates such a complication of bacillary dysentery —

Appamma, a Hindu married woman, aged about 25 years, was admitted into the Medical Wards of the King George Hospital, Vizagapatam, for swelling of the feet of 10 days' duration

Her *previous history* showed that she was passing loose motions, four per day, accompanied by pain in the lower abdomen for 6 months prior to her admission into the hospital

The *general condition* of the patient was very poor, all the visible mucous membranes were very anæmic, pyorrhœa alveolaris was present and the tongue was coated

On *physical examination* the circulatory system was normal, except for the hæmic murmurs present, the respiratory and nervous systems showed no abnormality The liver and spleen were not palpable There was no ascites

The blood showed signs of marked secondary anæmia, the blood Wassermann was weakly positive, the urine showed only a trace of albumin No amœbæ or cysts were found in the stools

The patient's condition gradually became worse and she died 23 days after admission

Autopsy There was no fluid in the peritoneal cavity Peritoneum was smooth and shiny Liver was smaller than normal and on histological examination showed early changes of multi lobular cirrhosis Mesenteric glands were enlarged Typical bacillary dysenteric ulceration of the descending and sigmoid colon was present and it was confirmed by histological examination

Comment Clinically it was not possible to diagnose cirrhosis of the liver, in this case Ascites might have developed if the patient had survived for a longer time

(*c*) The results of this clinical investigation confirm the views expressed in a previous paper that the commonest causes of ascites in this district are cirrhosis of the liver and decompensation of the heart

In the investigation of the cause of ascites, portal cirrhosis should not be excluded even if there is no history of excessive consumption of alcohol or if the patient survives several tappings In all the cases in which portal cirrhosis was verified post-mortem no history of excessive drinking of alcohol was obtained Hence, some other cause should be found to account for portal cirrhosis, which is

definitely proved from the post-mortem records of the Medical College Hospital (King George Hospital), to be the commonest type of cirrhosis met with in this district It may be that some toxin, closely allied to alcohol in its action, which was absorbed into the portal circulation from the intestinal tract, produced the portal cirrhosis in these cases The results of the investigation into the ætiology of portal cirrhosis in this district will be discussed in a separate paper

Because a patient survives several tapping, it need not be a point against the diagnosis of cirrhosis of the liver In most of the cases in whom the diagnosis of cirrhosis of the liver was definitely established, paracentesis of the abdomen had to be repeated a number of times to relieve pressure symptoms and was attended with no ill-effects In the patient in case No 8, in whom the diagnosis of portal cirrhosis was verified at operation, paracentesis was done 8 times during his 2 months' stay in the hospital Barker (1930*a*) has drawn attention to a patient 'who had to be tapped over fifty times for ascites due to portal cirrhosis'

The results of the clinical investigation clearly show that it is not always easy to arrive at the causation of ascites in every case A systematic investigation should be undertaken in all obscure cases, with a view to unravel the ætiologic factor Undue importance should not be attached to the history of any case, which should be considered along with the results of the physical and laboratory examinations In the light of modern biochemical methods an attempt should be made to diagnose ascites due to cirrhosis of the liver at an early stage, before all the classical signs develop The importance of this will be realized from the statement of Rowntree (1929) that in 75 per cent of patients suffering from portal cirrhosis, the ascites will yield to Merbaphen (Novasurol) or Mersalyl (Salyrgan) and ammonium salts

Summary and conclusions

(1) The results of the investigation into the cause of ascites in this part of the Madras Presidency definitely show that cirrhosis of the liver and decompensation of the heart are the commonest causes of ascites, and do not support the view of Megaw, that 'the commonest form of ascites in India is not due to cirrhosis of the liver but to a fibrosis of the peritoneum resulting from irritation of the peritoneum by the toxins of the bacilli of dysentery'

(2) Kala-azar is not seen here and does not play any rôle in the ætiology of ascites

(3) Bacillary dysentery is very common and the Flexner group of organisms appear to be the common invaders

(4) The rôle of bacillary dysentery in the causation of ascites is discussed It is shown that 'chronic dysenteric peritonitis' (Megaw) is rare and should not be considered as a 'common' cause of ascites, hepatic cirrhosis is the probable cause even in cases of ascites with previous history of dysentery

(5) While positive agglutination tests against the bacilli of Shiga and Flexner point to a previous infection with bacillary dysentery, they fail to prove the ætiologic relationship between dysentery and ascites A history of dysentery shortly before the onset of ascites is of no particular significance and it should not be taken as a point in favour of 'chronic dysenteric peritonitis' leading to ascites

(6) The causes of chronic peritonitis are many and it is not always easy to prove whether the thickening of the peritoneum is the cause or the result of ascites Even if evidences of bacillary dysentery, recent or old, are present in the intestines, the thickening of the peritoneum cannot be attributed to the dysentery, unless all the other causes for the change in the peritoneum are excluded

(7) A diagnosis of cirrhosis of the liver should not be excluded in cases of ascites, because there is no history of excessive drinking of alcohol or the patient has survived several tappings

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THE SEROLOGICAL CLASSIFICATION OF LACTOSE FERMENTERS (*B COLI*) AS A GUIDE IN DETERMINING THEIR SOURCE

BY

M R Ry Rao Bahadur T SITAPATI AYYAR Avl, l m & s,

Assistant Director, King Institute

(*From the King Institute of Preventive Medicine, Guindy*)

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Introduction

Much confusion exists in the nomenclature of the group of organisms commonly referred to as the lactose fermenters—more comprehensively called the colon group During the course of bacterial research on the fæces of newly born and entirely breast-fed children, Escherich found in 1885 that certain bacteria appeared in the fæces and were always present in the colon, either confined to the large intestines or at least not extending far beyond the ileo-cæcal valve into the small intestines Subsequent studies of the actions of the members of the group on carbohydrates, alcohols, and glucosides showed a very great difference in the individual members found Levine pointed out in 1918 that as the number of fermentable substances or other characters observed was increased, the number of varieties was given by the formula 2^n where n is the number of characters studied Thus with 8 characters there would be 256 possible combinations and the number would rise to 1,024 with 10 characters

From 1905 to 1909 MacConkey employed saccharose and dulcite for subdividing the group into four main divisions, each type included a number of varieties distinguished by gelatine liquefaction, indol production, Voges-Proskauer reaction, motility and fermentation of inulin and adonitol On this classification a system of bacteriological standards for tropical water-supplies was developed by Colonel Clemesha, Govindarajulu and the author of this paper and published as an appendix to the *Annual Report of the King Institute*, in 1908 There is no doubt that the fermentation reactions of the *coli* group are sufficiently constant to be of service in

the classification of its members Occasionally an organism on prolonged cultivation may lose its fermenting capacity, but such exceptions are not numerous enough to detract from the value of the method As a rule the carbohydrate is attacked promptly and acid and gas formed in less than 24 hours

It is of interest to trace the different tests introduced in determining the organism Thus Escherich in 1885 used motility and coagulation of milk as tests for the group Theobald Smith added capsule formation and saccharose fermentation in 1893 and the ratio of gases evolved in the fermentation of glucose in 1895 In 1901 Durham added starch and inulin to the list of fermentable substances and the reactions described by Voges-Proskauer In 1905 MacConkey used saccharose and dulcitol Harden and Walpole added a more accurate quantitative analysis of gas production in 1906 Clarke and Lubes in 1915 introduced hydrogen-ion concentration before and after fermenting glucose using methyl-red as an indicator He grouped the lactose fermenters into high and low ratio groups Kligler modified the MacConkey's classification by substituting salicin in place of dulcitol in 1914 and classified the organism into four groups (1) *acidi lactici*, (2) *B coli communis*, (3) *B coli communior*, (4) *B lactis aerogenes* He further used glycerine to divide the ærogenes group into *cloacæ* and *ærogenes* In 1913 fermentation of inositol was considered by Mackie as an important criterion in the grouping

Levine in 1916–18 studied *coli*-like bacteria isolated from soil, sewage and fæces as to their characters such as fermenting different materials—glucose lactose, saccharose, salicin, glycerine starch, liquefying gelatine, the presence or absence of motility and the double reactions indicated by the symbols M R and V P (methyl-red and Voges-Proskauer) He introduced the classification of the L F * into fæcal and non-fæcal or what he called 'high and low ratio organisms' In 1923 24 Stewart and Koser introduced a new test in citrate utilization by means of which a claim was made to distinguish the lactose fermenters of animal origin from those in soil In 1924 to 1926 Raghavachari in a study of the applicability of the two tests, viz, proportion of high and low ratio lactose fermenters and Koser's citrate utilization phenomenon applied to L F contained in Indian waters, found that no definite diagnosis as to *coli* of human, bovine or soil origin could be arrived at

Pawan (1931) has been exhaustively and critically reviewing this subject under the heading 'Some aspects of the Colon group of organisms' He has discussed the various standards used by bacteriologists from time to time as indications of human fæcal pollution He has also on occasion critically applied some of the methods to water samples from Trinidad The review is not yet completed So far his conclusions seem to point definitely to 'lack of correlation between the tests in existence and the sanitary qualities of waters'

* Throughout this paper the abbreviation 'L F' has been used to signify 'Lactose Fermenters'

From the above literature extending over a period of about five decades, it may be observed that the various biochemical tests employed were able merely to subdivide the lactose fermenters into more and more varieties without grouping them into any definite types Recent advances in bacteriology dealing with the serological behaviour of organisms, has definitely helped in the classification of organisms such as the enteric and dysenteric and food poisoning groups The serum test of agglutination was therefore considered suitable for grouping the lactose fermenters Before going into the method of the preparation of agglutinating sera against lactose fermenters, it is perhaps better to describe briefly the more important characteristics of those organisms and particularly those relating to their antigenic properties

Natural characteristics of lactose fermenters—Organisms of this group are found normally in the intestines of all warm-blooded animals and many cold-blooded creatures They have been isolated from grasses and grains In some instances they live a saprophytic life outside the bodies of animals and in others they are found associated with diarrhœa, peritonitis, pyelitis, cystitis and other inflammatory conditions They are found to show much variation such as the dissociation into smooth and rough hæmolytic and non-hæmolytic forms They ferment several sugars A constant characteristic of the group has, however, been found to be the fermentation of the sugar lactose and so the name 'Lactose Fermenters' has been appropriately given to this group of organisms

Antigenic value—Some of the organisms of the group have been found to possess antigenic value to a marked degree With the exception of a small experiment by Durham who found in 1897 that the serum of an immunized animal agglutinated the homologous strain to a high titre but other strains either not at all or only in low titre, it has been the opinion of numerous observers until recently that *B coli* was mainly saprophytic and could not develop agglutinins In 1921 Dudgeon and his colleagues showed that the hæmolytic strains of *B coli* met with in infections of the urinary tract were practically all agglutinated by the serum obtained from an animal immunized against a single strain, while the non-hæmolytic strains were separate entities since they were only agglutinable by homologous sera This was confirmed by Meyer and Homenby in 1924 In 1926 Hoes and Strunng independently proved by agglutination and saturation tests that a kinship existed between a number of strains isolated by them Besides the *principal agglutinins* developed by certain strains of *B coli* it was noted that there was a formation of a *partial agglutinin* for strains of *B coli* in the serum of animals immunized by *B typhosus* and *B dysenteriæ* Differing from the above two kinds of agglutinins there had been noted a third kind known as *heterologous agglutinin* (Nebenagglutinin) which was formed in certain infections as cerebro-spinal and typhoid fevers Kahn and his colleagues cultivated from the stools of dysentery patients *B coli* strains which agglutinated with the dysentery high titre serum, which phenomenon

was termed *para-agglutination* The reaction was explained by them by the fact that *B coli* by close association with the dysentery bacillus in the body of the patient had its reception apparatus modified to resemble that of the pathogenic organism This specific modification of the *coli* organism in dysentery stools was taken advantage of in the preparation of the special type of antigen used in obtaining agglutinating serum for the human type of *coli* organisms

Preparation of agglutinating serum against L F—The organisms were in the first place isolated from several specimens of dysenteric stools and normal specimens of calf-dung by the usual method of inoculating MacConkey's media To select a few predominating types from the large number of organisms they were further inoculated into sets of six common sugars, lactose, saccharose dulcite, inulin, adonite and raffinose By the fermentative action on these sugars the organisms were grouped into 30 combinations as shown in Appendix I The organisms thus limited in number by the sugar reactions, were then arranged in their order of predominance, as found in Appendix II recording results of specimens of intestinal contents of man, calf, rabbit, monkey and pigeon and in soils

In an experiment adopting the above technique 321 L F were isolated from 60 specimens of dysenteric stools and inoculated into the different sugars and classified into groups as shown in Appendix II The first ten predominating organisms were separated out, sub-cultured, emulsified, and mixed to constitute the antigen Each emulsion was further divided up into dead and living emulsions to be inoculated successively

From 46 specimens of calf-dung 192 L F were isolated and classified by sugar reactions into different groups, according to their predominance The first seven type organisms were sub-cultured and emulsified into a specific antigen for the bovine type of agglutinating serum as the organisms were tested and found to possess antigenic property The pooled emulsion was, as in the case of human strains, divided up into dead and living emulsions to be used in inoculating animals successively in the preparation of the homologous serum Appendix III records the types of organisms from the predominant groups that were used as antigens

An intravenous injection of 5,000 millions of the dead organisms of human or bovine origin was by experiment found to be too toxic and fatal to rabbits weighing about 250 grammes A smaller dose of about 500 millions was chosen for the initial injection, and a dose of 5,000 millions was gradually worked up to by weekly injections of doses increasing regularly by 250 millions The same animal was then inoculated with 2 or 3 doses of a live emulsion of 500 millions Small quantities of blood were taken from the ear veins of the treated animals and assayed for the content of agglutinins As soon as the titre was about 1,000 and upwards, the animals were bled and serum collected and stored in the usual way in capsules

Classification of L F into human, bovine and soil types by the use of the two specific sera prepared—The two types of polyvalent agglutinating sera prepared

as described above were then used to differentiate the L F into animal (human and bovine) and non-animal (soil) type The L F isolated from different specimens of normal fæces and calf-dung were then tested against the two types of agglutinating sera in the following way The organisms were primarily verified as to their characteristic fermentation of lactose They then were each emulsified in drops of the human and bovine high titre serum, kept on a slide and slightly rocked to and fro for a few minutes as is done in the technique of agglutination by the slide method The phenomenon of visible clumping was observed and noted The organisms were classified as human or bovine according to the agglutination caused by 1 in 50 dilutions of the respective sera Whenever the organism was affected by both the sera, end-points were worked out by using further dilutions of the sera and then the organisms were classified according to the serum which clumped the organisms in the highest dilution The organisms which could not be clumped by either serum in 1 in 50 dilution, were labelled as 'soil'—strictly this group should be called 'non-human and non-bovine', as possibly some may be derived from other animals but for convenience they will be called 'soil' type

TABLE I

Showing the percentage of human and bovine L F in the corresponding specimens

Nature of examination	Human faces	Calf dung	Sewage
Total number of specimens examined	62	58	29
Number of specimens in which corresponding type of L F were present	57 showing human type of L F	58 showing bovine type of L F	25* showing human type of L F
Percentage of specimens in which corresponding type of L F were present	92 showing human type of L F	100 showing bovine type of L F	86 showing human type of L F

* In the four negative specimens of sewage only 4, 6, 3 and 5 lactose fermenters respectively could be obtained for examination as the samples of sewage were very dilute

From each of 62 specimens of human fæces and 58 specimens of cow-dung 10 colonies of L F from unselected areas of growths on MacConkey plates were examined against human and bovine agglutinating sera and the results are recorded in the above table From the table it will be found that 57 out of 62 specimens of fæces, that is a percentage of 92, contained human type of L F and cent per cent of cow-dung specimens contained bovine type of L F In specimens of sewage 25 out of 29 specimens, i e , 86 per cent contained human type The four specimens

of sewage which were negative were very much diluted, as the specimens contained few L F It will be seen from the above facts that every specimen of cow dung examined showed bovine type of L F and nearly 9 out of 10 specimens of human faeces and sewage showed human type of L F, conclusively proving that presence of human or bovine type of L F forms a characteristic of human fæces and cow-dung respectively

Appendix IV contains the results of agglutination of L F by the two types of sera and classification into human, bovine and soil types From a study of the figures in Appendix IV it is very interesting to note that only 34 per cent of the total 628 lactose fermenters isolated from 62 specimens of fæces, were classified as human and bovine in origin while the bulk of the L F were of soil type Of the 581 L F isolated from specimens of calf-dung nearly 70 per cent were of human and bovine origin, while a small percentage of 30 proved to be of soil type Of the L F from human fæces that were of animal (human and bovine) origin, 77 per cent were agglutinated by the human type serum and the rest by the bovine type It was thus found that in fæces there was a preponderance of the human type, to the extent of about 3½ times the number of bovine species Of those organisms marked out as of animal (human and bovine) origin from the specimens of calf-dung, 92 7 per cent worked out as bovine type, thus preponderating to the extent of about 13½ times the human type The finding of human or bovine types of lactose fermenters in high proportions in the corresponding specimens form valuable evidence in support of the serological classification

Next, 162 specimens of earth were collected from different soils and their bacterial flora was studied Definite weighed quantities of specimens of soil were put in MacConkey broth and loops from the fermented tubes were plated on bile salt-lactose-agar plates Red colonies were picked out and tested in lactose sugar media The L F thus identified were tested against two types of sera as described in the previous experiment It is interesting to note that many samples of earth did not yield L F In Appendix IV are recorded agglutination results of 374 lactose fermenters isolated from several samples of soils, 97 per cent of them could not be agglutinated by the human or bovine types of sera and should be taken specifically as non-animal or soil type Thus in soils there was a preponderance of the soil type of L F to the extent of 33 times the human and bovine types From these figures we can infer that most L F not clumped by human or bovine types, form a separate group which is to be styled as 'soil type'

Application of the serological method to specimens from sources other than human and bovine—In another set of experiments 28 samples of sewage comprising house and latrine washings collected from cess-pools, were examined bacteriologically Small quantities of the sewage were inoculated into bilesalt-broth and loops from the fermented broths were plated on MacConkey agar and 252 lactose fermenters were isolated Then agglutination tests against the two types of sera are also

recorded in Appendix IV The findings, as one would expect, are similar to those of human fæces, 37 5 per cent being classified as of human and bovine origin of which no less than 91 5 per cent belonged to the 'human type' Excreta from pigeons, rabbits and monkeys were also subjected to similar analysis Table II describes the results of serological classification with different strains of lactose fermenters obtained from specimens of human fæces, calf-dung, soils, sewages and also the evacuations of rabbits, monkeys and pigeons From the last 3 columns, dealing with the L F contained in the evacuations of the last three animals, it is interesting to note that 96 to 98 per cent of the organisms belong to the so-called 'soil type' As regards this we must remember that the classifying of organisms as being of the soil type is simply the result of a *negative* reaction to sera against human and bovine strains, so here the 'soil' type is simply a general group and includes organisms derived from sources other than human and bovine We should expect that the lactose fermenters would fall into the general undifferentiated non-human non-bovine group which is called the soil group

From Table II it is seen that lactose fermenters of the human type were present in high ratios in human fæces and sewage, L F of bovine type in very high ratio in calf-dung and L F of soil type, also in very high ratio, in soils and evacuations of small animals such as the rabbit, monkey and pigeon

Application of the new classification of lactose fermenters to water samples— The new method of classifying L F was then applied in assaying the quality of water samples The presence of human types would definitely indicate contamination with sewage or human fæces Such waters would be liable to contain organisms of dangerous water-borne diseases such as cholera, dysentery or typhoid and should be classified as bad The presence of bovine type in a sample would point to the water having had direct contamination with cattle excreta As they would be liable to have had access to human washings as well on occasions, they should be treated as highly suspicious and kept under observation Presence of soil types alone should not eliminate a sample from being potable

The technique of analysing a water with a view to adapting the results to the new classification becomes simplified Different quantities of water samples are inoculated into bilesalt-broths and incubated at 37°C At the end of 48 hours loopfuls from the fermented tubes are smeared out on MacConkey plates and incubated for a day L F colonies, about 10 to 20 from an unselected area from the plate, should then be picked out, cultured, tested against the two different types of sera and classified into human, bovine or soil According to the nature of the organisms present the quality of water can be determined

In Appendix V are recorded the results of analysis of 812 water samples which were brought in for routine examination at the institute The findings of the serological, bacteriological and chemical examinations are recorded side by side for comparison For convenience in comparing, the samples have been classified

TABLE II

Showing percentage classification of L F of human, bovine and soil types in different sources.

Nature of material examined	FÆCES (HUMAN)		CALF-DUNG		SOILS		SEWAGE		RABBIT DUNG		MONKEY EVACUATIONS		PIGEONS' DROPPINGS	
	Total organisms	Percentage	Total organisms	Percentage	Total organisms	Percentage	Total organisms	Percentage	Total organisms	Percentage	Total organisms	Percentage	Total organisms	Percentage
Total organisms isolated	628 (L F only selected)		581 (L F only selected)		504		252 (L F only selected)		196		173		172	
Non lactose fermenters	*Nil*		*Nil*		130		*Nil*		4		8		3	
Lactose fermenters	628		581		374		252		192		165		169	
Soil type	415	66	174	30	363	97	158	62 5	184	95 8	162	98	165	98
Human and bovine	213	34	407	70	11	3	94	37 5	8	4 2	3	2	4	2
(*a*) Human type	164	77	30	7 3	9		86	91 5	5		2		1	
(*b*) Bovine type	49	23	377	92 7	2		8	8 5	3		1		3	

into good, suspicious and bad according to the more or less accepted standards of each method which we may roughly describe as follows —

In the serological method, a sample which contains human types of L F is classified as bad, while the presence of bovine type indicates liability to pollution, and the presence of soil type alone without any L F of animal type indicates that the water could not be condemned In the bacteriological method the absence of L F in quantities below 5 c c and total counts not more than 1,000 per c c are chiefly looked for in good potable waters, L F in 1 c c and total counts of about 2,000 per c c indicate that the water is liable to contamination, while the presence of L F in quantities of water below 1 c c with a total count of over 2,000, point to the fact that the water is grossly contaminated In the chemical analysis ammoniacal nitrogen less than 0 002 part, oxidized nitrogen less than 0 01 part and oxygen absorption less than 0 1 part per 100,000 and the absence of nitrites, would indicate good or potable waters If the figures are higher the samples are suspicious If in addition nitrites are present, samples should be looked upon as bad or polluted

Comparative study of the chemical, bacteriological and serological methods in assaying the quality of waters —Of the 812 samples of water analysed at the institute specially with this view, 165 were found not suitable for comparison as no lactose fermenters could be obtained in about 60 c c of the samples Of the remaining 647 samples, in 65 per cent agreement was shown in the results of the serological method with those of either or both of the other methods The results as well as the standards described above are given in Appendix V

The serological method proved itself of the utmost value as compared with the other methods in vogue in the following interesting experiment The experiment was conducted by Dr J F Kendrick of the Rockefeller Foundation to find out if intestinal parasites such as hookworm and their larva and ova, could pass through a certain distance of soil to contaminate water sources He dug out in the centre of a field a large pit wherein he dumped fæcal rubbish which was kept moist throughout the experiment At a distance of 20 feet from the pit radially he put up four boreholes Samples of water from each of the four boreholes surrounding the dumping pit containing fæces were analysed by the institute by the three methods, serological, bacteriological and chemical, at weekly intervals for a period of 7 months The results are presented in a tabular form according to the standards above described for each of the method On 24 occasions a sample was taken from each of the four boreholes By the serological method definite signs of fæcal pollution were made out in all the boreholes within a week of starting of the experiment as indicated by the presence of L F of human type No such definite indication could be brought out by the other two methods It is interesting to note from the table that the definite sign of human fæcal pollution by the serological method was obtained 4 times in borehole No 1, 8 times in No 2, 7 times in No 3, and thrice in No 4 The results are recorded in Appendix VI

SUMMARY

1 The term '*Bacillus coli*' or 'Colon group' does not express with any definiteness the nature of the organism A great number of organisms fall in the group, their numbers increasing with the number of tests used As the common and constant characteristic of the organisms is the fermentation of the sugar lactose, the term 'Lactose Fermenters' (L F) has been used in the paper to denote the group

2 A number of L F when in symbiosis with the pathogenic organisms of dysentery acquire a high antigenic property, and the capacity to give rise to an agglutinating serum specific in nature They fall serologically into a type conveniently labelled as human

3 Similarly a definite number of L F can be isolated from calf-dung which can cause the production of a specific agglutinating serum, capable of agglutinating the homologous bovine type of L F

4 A very large percentage of L F are found in soils and grains which are not clumped by either of the above-mentioned specific sera of human or bovine types Such organisms are classified into a general type called 'soil'

5 By the application of the serological method to the L F isolated from normal specimens of intestinal excreta of men and cattle and from soils, homologous types of organisms were found in high ratios in the respective specimens, 77 per cent were of human type in fæces, 92 per cent were bovine type in cattle dung and 97 per cent were of soil type in soils The human type preponderates about 3½ times over the bovine in human fæces, the bovine type preponderates about 13½ times over the human in cattle dung and the soil type about 33 times over those of animal origin in soils

6 In samples of sewage the L F are found to be classified by this method more or less in the same way as in human excreta 37 5 per cent are of animal origin of which 91 5 per cent are of human type

7 In the excreta of small animals such as rabbits, monkeys and pigeons, 95 to 98 per cent of lactose fermenters fall in the general category 'soil type

8 The serological classification of L F into human, bovine and soil types can be applied in assaying the quality of waters more usefully than the bacteriological and chemical methods in vogue It has been found that an agreement existed in 65 per cent of samples between the findings by the serological method and those of either or both of the other methods

9 In an experiment to find out if contamination from dumped fæcal matter can be transmitted to sub-soil water through a distance of about 20 feet, the serological method alone has been useful in demonstrating the presence of L F of human type within a week while the other methods failed to prove definitely the transmission of fæcal contamination

In conclusion I have very great pleasure in recording my thanks to Colonel H H King, I M S, Director of the King Institute, Dr C G Pandit, M B, B S, Ph D, D P H & D T M, First Assistant Director, King Institute, and Major W J Webster, M C, M D, I M S, for their valuable suggestions in the preparation of this paper

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Appendix I

Thirty possible combinations into which lactose fermenters group by biochemical reactions on six important sugars

Lactose	Saccharose	Dulcite	Inulin	Adonite	Raffinose	Group
+	−	−	−	−	−	1
+	+	−	−	−	−	2
+	−	+	−	−	−	3
+	−	−	+	−	−	4
+	−	−	−	+	−	5
+	−	−	−	−	+	6
+	+	+	−	−	−	7
+	−	+	+	−	−	8
+	−	+	+	+	−	9
+	−	−	−	+	+	10
+	+	+	+	−	−	11
+	−	+	+	+	−	12
+	−	+	+	+	+	13
+	+	+	+	+	−	14
+	−	+	+	+	+	15
+	+	+	+	+	+	16
+	+	−	+	−	−	17
+	+	−	−	+	−	18
+	+	−	−	−	+	19
+	+	−	−	+	+	20
+	+	−	+	−	+	21
+	+	+	−	+	+	22
+	+	+	−	−	+	23
+	+	+	+	−	+	24
+	−	+	−	+	+	25
+	−	+	−	−	+	26
+	−	+	−	+	−	27
+	−	+	+	−	+	28
+	−	−	++	+	+	29
+	−	−	+	−	+	30

Appendix II.

Classification of L F present in fæces, calf-dung, soil and in excreta of rabbit, monkey and pigeon according to their predominating numbers as per classification of Table I

Dates of work.	Human fæces			Calf dung			Soil			Serial number	Rabbit excreta			Monkey excreta			Pigeon droppings		
	Number of L F	Group	Names	Number of L F	Group	Names	Number of L F	Group	Names		Number of L F	Group	Names	Number of L F	Group	Names	Number of L F	Group	Names
From 15-1-25 to 4-4-25	61	23	*B neapolitanus*	92	26	*B coli communis*	95	2	*B coscoraba*	1	83	2	*B coscoraba*	50	2	*B coscoraba*	58	1	*B vesiculosus*
	49	1	*B vesiculosus*	25	23	*B neapolitanus*	95	19	*B* 106	2	31	1	*B vesiculosus*	38	6	"	53	2	*B coscoraba*
	46	3	*B schæfferi*	22	6	*B* "	39	1	*B vesiculosus*	3	21	21	"	29	1	*B vesiculosus*	12	17	*B* 105
	41	26	*B coli communis*	18	1	*B vesiculosus*	29	1	*B* 10	4	17	17	*B* 105	17	19	*B* 106	12	21	*B* ?
	25	25	*B* 33	9	3	*B schæfferi*	23	6	?	5	17	19	*B* 106	8	21	"	11	19	*B* 106
	20	6	?	9	19	*B* 106	15	7	*B neapolitanus*	6	11	1	*B* 10	6	1	*B* 10	5	30	*B levans*
	17	19	*B* 106	9	25	*B* 33	11	21	?	7	5	6	?	3	30	*B levans*	3	1	*B* 10
	16	22	*B rhinoscleroma*	7	22	*B rhinoscleroma*	12	17	*B* 105	8	5	30	*B levans*						
	9	5	*B* 9				11	3	*B schæfferi*	9	2	20	?						
	7	2	*B coscoraba*				7	11	*B* 70	10									
	7	7	*B neapolitanus*				7	30	*B levans*	11									
	6	27	*B* 33 ?				1	20	?	12									
	4	24	*B* 69				1	24	*B* 69	13									
	3	18	*B gasiformans*				1	26	*B coli communis*	14									
	3	25	*B* 33 ?							15									
	1	4	*B* 10 ?							16									
	1	10	?							17									
	1	10	?							18									

Appendix III

List of selected types of L F from each of the predominant groups found in human fæces and calf-dung of Table II, that are used in the specific antigens in the preparation of high titre sera

Serial number	Source	Lactose	Saccharose	Dulcite	Inulin	Adonite	Raffinose	Group	Names as per classification of MacConkey
1	Bovine	+	−	+	−	−	+	26	*B coli communis*
2	,,	+	+	+	−	−	+	23	*B neapolitanus*
3	,,	+	−	−	−	−	+	6	?
4	,,	+	−	−	−	−	−	1	*B vesiculosus*
5	,,	+	−	+	−	−	−	3	*B schæfferi*
6	,,	+	+	−	−	−	+	19	*B* 110
7	,,	+	−	+	−	+	+	25	*B* 33
1	Human	+	+	+	−	−	+	23	*B neapolitanus*
2	,,	+	−	−	−	−	−	1	*B vesiculosus*
3	,,	+	−	+	−	−	−	3	*B schæfferi*
4	,,	+	−	+	−	−	+	26	*B coli communis*
5	,,	+	−	+	−	+	+	25	*B* 33
6	,,	+	−	−	−	−	+	6	?
7	,,	+	+	−	−	−	+	19	*B* 106
8	,,	+	+	+	−	+	+	22	*B rhinoscleroma*
9	,,	+	−	−	−	+	−	5	*B* 9
10	,,	+	+	−	−	−	−	2	*B coscoraba*

Appendix IV

Comparative statement showing the percentage findings of the three types of L F. (human, bovine and soil) present in different specimens of fæces (human) calf-dung and soil as differentiated by the serological test

Lactose fermenters with various end-points of agglutination	Dilution of human and bovine agglutinating sera										L F in specimens of human fæces			L F in specimens of calf dung			L F in specimens of soil			L F in specimens of sewage			L F in specimens of rabbit excreta			L F in specimens of monkey excreta			L F in specimens of pigeons excreta		
	50		100		200		300		400		Positives	Total	Per cent	Positives	Total	Per cent	Positives	Total	Per cent	Positives	Total	Per cent	Positives	Total	Per cent	Positives	Total	Per cent	Positives	Total	Per cent
	H	B	H	B	H	B	H	B	H	B																				
Human type	+	−									108	164	77	30	30	7	5	6	?	0	86	91·5	1	5		2	3		0	0	
	+	+	+	−							32			0			1			81			2			1					
	+	+	+	+	−	−					13			0			0			1											
	+	+	+	+	+	−	−	−			11			0			0			4											
	+	+	+	+	+	+	−	−	−	−	*Nil*			0			0			0											
Bovine type	−	+									42	49	23	312	376	93	1	2	?	8	8	8·5	1	3		1	1		3	3	
	+	+	−	+							3			32			0			0			2			0					
	+	+	+	+	−	+					2			16			1			0						0					
	+	+	+	+	+	+	−	+			0			11			0			0						0					
	+	+	+	+	+	+	+	+	−	+	2			5			0			0											
Animal origin of L F and percentage in total												213	34		106	70		11	3		91	37·5		8			1			3	
Soil type												415	66		174	30		363	97		153	62·5		184			162			165	
Non lactose fermenters as rejected												*Nil*			*Nil*			130						1*			8*			3*	
Grand Total												628			580			504			252			196			174			171	

* As causing auto agglutination

Appendix V

Comparative statement of results of analysis of water samples by the three methods (1) bacteriological, (2) chemical, and (3) serological classification of L F

Recognized standards used in the three methods for the quality of waters

Quality of waters	Bacteriological method	Chemical method	Serological method
Good or potable waters	Total count less than 1,000 Presence of L F in 5 c c and upwards	Presence of NH_4 in 0 001 parts Nitrates in 0 01 Oxygen absorption in 0 1 parts	Absence of human or bovine L F
Suspicious	Total counts of 2,000 to 5,000 Presence of L F in 1 c c and upwards	Presence of NH_4 in 0 002 parts Nitrates in 0 02 to 0 05 Oxygen absorption 0 2 to 0 5 parts	Presence of bovine L F only
Bad or unfit for consumption	Total count of 5,000 to countless Presence of L F in 0 01 or 0 1 c c and upwards	Presence of NH_4 in 0 005 to 0 01 Nitrates in 0 05 to 0 1 Oxygen absorption 0 5 to 1 Presence of free nitrogen as nitrites in traces	Presence of human L F

1	Total water samples analysed	812
2	Samples unsuitable for comparison due to absence of L F in 60 c c	165
3	Samples in which all the figures of the 3 methods are available for comparison	647
4	Samples in which there is agreement of all the three methods	168
5	Samples in which there is agreement between serological and bacteriological	126
6	Samples in which agreement exists in serological and chemical	125
7	Total samples in which serological method agrees with either chemical or bacteriological or both	419
8	Percentage of samples in which agreement exists between serological and one or both of the other methods	65
9	Table showing classification of water samples according to their quality as indicated by the different methods	

Nature of method	Good	Percentage	Suspicious	Bad	Total	Percentage	Grand total of samples
Serological	329	50	166	163	329	50	658
Chemical	289	50 8	157	122	279	49 2	568
Bacteriological	369	56	168	121	289	44	658

Appendix VI

*Results of the experiment to prove the transmission of faecal contamination**

Date	Number of water sample	Borehole No 1			Borehole No 2			Borehole No 3			Borehole No 4		
		Good	Suspicious	Bad	Good	Suspicious	Bad	Good	Suspicious	Bad	Good	Suspicious	Bad
3-7-29	122	sbc			bc		s	bc		s	s	bc	
17-7-29	165	bc		s	c	b	s		bc	s	c	sb	
1-8-29	182	sc	b		bc			bc	s		sb	b	
9-8-29	210	bc	s		sbc			bc		s	c	b	s
15-8-29	227	bc	s		b		sc	bc		s	sc	b	
22-8-29	248	bc	s		No L F			sbc				bc	s
29-8-29	271	sbc			bc		s	No L F			c	sb	
5-9-29	304	No L F			No L F						sbc		
21-9-29	342	c	b	s	bc		s	c	b	s	c	sb	
26-9-29	372	sc	b		No L F			bc		s	c	sb	
3-10-29	374	sc	b		bc		s	sc	b		c	sb	
10-10-29	384	bc	s		bc		s	bc		s	s	bc	
17-10-29	408	bc		s	bc		s	bsc			bsc		
23-10-29	430	sb	c		sc	b			*			*	
13-11-29	196	sc	b			sbc			No L F			No L F	
20-11-29	518		sbc			sbc			No L F		bc		s
27-11-29	555		sbc			sbc			No L F			No L F	
4-12-29	595		sbc		sc	b			No L F		bc	s	
11-12-29	619	sbc			sbc			sbc				No L F	
18-12-29	653	sbc			sbc				No L F		sbc		
8-1-30	682	No L F			No L F				No L F		bs		
15-1-30	695	bc		s	sbc				No L F			No L F	
22-1-30	723	sbc			sbc			sbc			sbc		
12-2-30	780	sbc			sbc			sbc			sbc		

* Water samples from boreholes in the compound of Surgeon General's office surrounding a central pit in which artificially faecal rubbish was dumped

Note.—*s* denotes serological method *b* denotes bacteriological method *c* denotes chemical method

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STUDIES ON INDIAN SIMULIIDÆ

Part IV*.

DESCRIPTIONS OF TWO NEW SPECIES FROM NORTH-EAST INDIA *SIMULIUM HOWLETTI* SP N AND *SIMULIUM HIRTIPANNUS* SP N, WITH A NOTE ON *S ORNATUM* MEIGEN

BY

I M PURI, M SC (Punjab), PH D (Cantab), F E S,

In-Charge, Inquiry on Indian Simuliidæ, Culicoides and other Blood-Sucking Midges

(*From the Central Research Institute, Kasauli*)

[Received for publication, May 25, 1932]

THE species dealt with in this paper belong to the sub-genus *Simulium*, with the basal section of radius bare, front tarsi flattened, tergites of abdominal segments 6–8 large and shining and the claws of the female with a small sub-basal tooth They also belong to the same separate group as all those species described previously in Parts I to III of this study, being characterized by having a large conspicuous silvery white spot on the anterior surface of fore tibiæ

Simulium (Simulium) howletti SP N

FEMALE

Head dark grey, with fine golden hairs on the occiput, present though scanty on the face and a few on the frons along its lateral borders Frons dull dark grey, appearing somewhat shining in certain lights, comparatively small in size, about as high as its greatest width at the top, nearly parallel-sided, only very little

* In Parts I to III of this study, wherever the 8th abdominal sternite of the female has been described, the anterior border has wrongly been mentioned as the posterior and vice versa

narrowed in the region of the antennæ Face dusted with ash grey Antennæ brownish black on the two basal segments, the rest nearly black,* with very fine pale pubescence Palpi black, the various segments of the usual proportions

Thorax —Mesonotum covered with very fine golden pubescence, partly rubbed off in the type specimen integument dull When viewed from in front (Plate XV, fig 1) it shows a fairly wide black anterior and lateral border ending a little in front of the wing, the former broken in the middle, a pair of large somewhat rectangular silvery white lateral spots gradually fading away posteriorly about the region of the wing-bases, and separated in the middle by an olive black stripe moderately broad, beginning from the anterior end and gradually widening out posteriorly, the silvery grey spots extending forwards along the edge of the olive black stripe and to some extent also along the anterior border of the mesonotum † The olive black stripe shows three faint very narrow ash grey lines, one median and two lateral running along its edge, the rest of the mesonotum appears greenish black As usual when viewed from behind the black and grey colours are reversed The black border becomes silvery grey and silvery spots change to black while the rest of the mesonotum becomes pale grey Scutellum brownish black covered with coarse golden pubescence (rubbed off in the type) and a fringe of long black hairs Pleuræ dark slate with ash grey reflections, membranous area bare

Abdomen brownish black, the basal scale brownish with the long hairs golden, the dorsum of segment 2 with the usual greyish sheen Tergites of segments 3–5 small, rounded, dull, those of segments 6–8 very large, shining brown, sparsely covered with fine golden hairs *Terminalia* (Plate XV, fig 2) somewhat resemble those of *griseifrons* The posterior half of the ventral surface of segment 7 is uniformly covered with a large number of comparatively short macrosetæ Sternite of segment 8 fairly large, somewhat narrowed laterally, anterior border rounded, with a number of fairly long macrosetæ on the lateral portions, the middle one-third bare Anterior gonopophyses fairly large, covered practically all over with micro- and macrosetæ, their internal border slightly curved, thickened and produced slightly inwards near their posterior end Paraprocts large, strongly chitinized, cerci comparatively long

Legs —Fore coxæ and trochanters yellowish brown, the former show slight pale grey dusting in certain lights and the latter are dark grey distally, femora dark

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* The colour of the antenna does not seem to be a constant character in this species In a paratype specimen in which only the basal portion of antennæ are present the two basal segments, and the base of segment 3 are yellowish brown and the rest (as much of it as is present) is dark brown

† In some paratype specimens the lateral spots extend much more along the anterior border than in the type specimen so that the black horizontal stripe lies a little behind the anterior border and is crescent shaped

brown with a somewhat pale base, tibiæ pale yellow with a dark brown base and distal one-fourth nearly black, the anterior surface with a large silvery white spot and the posterior border brownish, tarsi black, moderately flattened, the first segment about 5 times as long as its greatest width at the distal end Middle and hind coxæ brownish black, trochanters and femora dark brown, the latter yellowish basally and nearly black near the tip Middle tibiæ pale yellow with whitish sheen on the posterior surface, the distal one-fourth nearly black, basal half of the first tarsal segment yellowish, the rest of tarsi black Hind tibiæ brownish yellow on the basal two-thirds, distally becoming brown to nearly black on the distal one-fourth, with a whitish sheen on the posterior surface of the basal three-fourths, first tarsal segment pale yellow gradually becoming black on about the distal half and with its anterior border black, basal half of segment 2 yellowish, the rest of tarsi black Except the black portions, the legs have a fine golden pubescence Pedisulcus and calcipala well marked All claws with a small sub-basal tooth

Wing —Normal, hyaline, radius bare as far as the fork, radial sector a concave vein Wing length 4 2 mm The average length of wings of five specimens 4 0 mm Halteres orange

The ventral surface of the buccal cavity bears a large cluster of minute nodules at its distal end

MALE

Head black, with a fringe of short black hairs on the occiput Face with ash grey reflections, and sparse black hairs along its lateral borders Antennæ black with very fine pale pubescence, basal segment brownish black Palpi black

Thorax —Mesonotum velvet black, covered fairly densely with a coarse pale golden pubescence—mostly rubbed off in the type In the fore corners is a pair of large silvery grey spots, narrowly or broadly separated from each other in the middle, like those found in *S ornatum* Meigen, only about half of each of these spots reflects light at one time (In one paratype specimen even the portion between the two spots appears silvery grey) In certain lights the lateral and hind borders of mesonotum are also silvery grey Scutellum brownish black, covered with coarse golden pubescence* and a fringe of long black hairs Pleuræ slate black or brownish black, with ash grey reflections, membranous area bare

Abdomen velvet black, with scattered fine golden hairs dorsally and clusters of long and somewhat paler hairs laterally, long hairs on basal scale brownish

* Partly rubbed off in the type

golden, segments 2 and 5–7 with the usual silvery grey spots *Genital armature* (Plate XV, fig 3) Coxites are somewhat broader than their length. Styles appear massive and comparatively very broad They have a characteristic shape (Plate XV, fig 4), to some extent resembling that in *S barraudi* They are of a more or less uniform width up to about the middle in which region they broaden out, the outer border being produced upwards as a broadly rounded projection Beyond this they are slightly narrowed again and have a uniform breadth up to the tip Their length is nearly three and a half times as long as their breadth near their proximal end Near the base, their dorso-internal surface is produced into a small protuberance bearing a cluster of strong setæ The usual small subterminal spine is placed comparatively further away from the distal end of the styles The inter-coxal piece (Plate XVI, fig 5) has a moderately broad base from which a flattened keel-like process, whose surface is raised into two rows of rounded teeth-like protuberances along its lateral borders, is produced downwards It is continued forwards as a somewhat smooth and thinly chitinized plate with its lateral edges broken up into strong backwardly directed spines which are also present on the dorso-anterior surface of the keel-like process The clusters of spines in the region of the genital opening are comparatively small

Legs —Fore coxæ brown, the others brownish black, all trochanters and femora dark brown, the lateral nearly black distally Fore tibiæ pale yellow, dark brown basally and along its posterior border, the distal one-fifth black, the outer surface with a large silvery white spot, tarsi black, somewhat flattened, first segment nearly six times as long as its greatest width near its distal end Middle tibiæ yellow on the basal one-third, gradually becoming dark brown distally with the tip nearly black and the posterior surface with a whitish sheen on the basal one-third or so , basal one-third of the first tarsal segment yellowish brown, the rest of tarsi black Hind tibiæ brownish yellow basally, gradually becoming dark brown on the distal two-thirds to nearly black at the tip, the posterior surface of basal one-third with a whitish sheen, basal half of the first and the base of the second tarsal segments brownish yellow, the rest of tarsi black Basitarsus of the hind leg (Plate XVI, fig 6) somewhat enlarged Its length is 0 8 of that of the hind tibiæ and its greatest width (a little proximal to its distal end) is relatively more than that of the tibia being 0 27 of its own length while that of the tibia is only 0 24 All legs bear a fine golden pubescence more marked on yellowish parts than on others

Wing same as in female Wing length about 4 1 mm , average about the same as in female Halteres brown

Described from 5 females and 3 males most of them in a damaged condition All these specimens form part of the collection of Simuliidæ received from the Imperial Entomologist at the Imperial Institute for Agriculture, Pusa (Bihar,

India), and were collected by thelate Mr F M Howlett, from 'Nekty Bridge,* Pusa, Behar, 27-5-07'

Types for the present in my own collection

I have great pleasure in naming this species after the late Mr F M Howlett, Imperial Pathological Entomologist, who collected these specimens

Simulium (Simulium) hirtipannus SP N

FEMALE

Head black with short black hairs on the occiput, a few present on the face and also along the lateral borders of the frons Frons black, markedly shining, comparatively very wide, nearly half the width of the head, somewhat parallel-sided, very little narrowed in the region of the antennæ, about as long as broad near the top Face also comparatively broad, dull, dusted with ash grey † Antennæ with the scape, one basal flagellar segment and the base of the second flagellar segment brownish yellow, the rest of the antenna nearly black, with pale white pubescence Palpi nearly black

Thorax —Mesonotum black, markedly shining with a slight metallic tinge, appears slightly dusted with pale grey in certain lights, sparsely covered with very fine short hairs, mostly rubbed off in the type but present in the paratype specimens Scutellum black with black marginal hairs, a few present also on the prescutellar area Pleuræ dull slate black, membranous area bare

Abdomen black, practically bare, fringe of hairs on the basal scale fairly short, somewhat golden black Second tergite with the usual greyish reflections, tergites of segments 6–8 very large, black and shining, those of 6 and 7 bare, while that of 8th with a few black fairly long setæ laterally *Terminalia* (Plate XVI, fig 7) The ventral surface of segment 7 bears on its median one-third a patch of fairly long and thick black setæ which split into 3–6 branches a little above their base (Plate XVI, fig 8) External to this patch the macrosetæ are scattered and simple A few split setæ may also be present in a similar position on segment 6 Sternite of segment 8 is broad with comparatively short narrowed and somewhat rounded lateral ends and bears a few fairly long macrosetæ postero-laterally The rest of the sternite is bare, except for a few short macrosetæ present along the posterior border Anterior gonopophyses widely separated, their interno-posterior end broadly rounded and their inner border not thickened Each bears a more

* The label on each of these specimens shows the name of the locality as 'Nethy Bridge' but in a recent communication to me by the Imperial Entomologist he says "There is no place near about Pusa known as 'Nethy Bridge' but there is one called 'Nekty Bridge' which is about a mile to the north east of Pusa" In view of this definite communication the change from Nethy Bridge to Nekty Bridge has been made The former may be the name which was in use for the same locality in 1907, when the late Mr Howlett collected these specimens

† Cf *S nitidithorax*

or less horizontal row of fairly strong macrosetæ about its middle, the anterior part covered with microsetæ while the posterior, which from its colour appears more strongly chitinized, is bare Paraprocts are thinly chitinized and poorly developed, and the cerci too are comparatively small

Legs—Fore coxæ and trochanters yellow, the latter dark distally; femora blackish yellow, gradually becoming dark in the distal half to nearly black at the tip, tibiæ pale yellow with a large silvery white spot on the outer surface and with nearly black base and distal end, tarsi black, flattened, first segment only about 4 times as long as its greatest width near the distal end Segments 1–3 with the usual pair of long black hairs sub-apically on their posterior border Middle and hind coxæ trochanters and femora black, the latter two slightly yellowish basally, tibiæ also black, yellowish near the base, the basal half with a silvery sheen on the posterior surface Basal three-fourths of the first and basal half of the second tarsal segments of middle and hind legs and the base of the third segment of the middle legs yellow, the rest of tarsi black Calcipala well marked, extending up to the pedisulcus which is well defined All claws with a small sub-basal tooth Fore femora and the yellow parts of the legs with fine golden pubescence

Wings—Normal, hyaline, radius bare up to the fork, radial sector a concave vein Wing length about 2 4 mm Halteres yellow

The ventral wall of the buccal cavity at its posterior end is produced into the pharynx as a small triangular piece the distal half of which is covered with minute spinous processes, a few nodules are also present on each side a little external to the triangular piece Furca and spermatheca of the usual form and colour

MALE

Head black with a fringe of short black hairs on the occiput Face dusted with whitish grey, with scanty black hairs Antennæ nearly black, covered with a very fine pale pubescence

Thorax—Mesonotum velvet black, sparsely covered with very fine copper coloured pubescence In front are a pair of large elongated shimmering silvery spots which are seen complete and extend posteriorly as a broad silvery lateral border up to a little in front of the wing-bases In certain lights the posterior fourth of the mesonotum is also grey with a metallic sheen and is shining Scutellum velvet black with black marginal hairs Pleuræ black with a greyish sheen, membranous area bare

Abdomen velvet black with scattered very fine dark pubescence, fringe of long hairs on the basal scale black, segments 2 and 5–7 with the usual silvery spots *Genital armature* (Plate XVI, fig 9) Coxites about as long as they are broad The styles are moderately long (about three times as long as their greatest breadth) They are practically of uniform width in the basal region, broadening out only slightly about a third of their length from the base, posterior to this they are

comparatively much narrowed and uniformly broad up to the rounded distal end On the interno-dorsal surface of the region of their greatest width each of them bears a number of close-set, short setæ directed inwards The inter-coxal piece (Plate XVI, fig 10) has a broad base with rather stout lateral limbs From the base a fairly broad process of uniform width projects downwards and curves a little forwards This process has a somewhat truncated distal end and bears on its flattened posterior and ventral surface two lateral rows of three or four fairly strongly chitinized teeth, while a number of short setæ are present on its dorsal and anterior surface

Legs —Fore coxæ blackish yellow, trochanters and femora yellowish black, distal halves nearly black, tibiæ black with a large silvery white spot on the outer surface, tarsi black, considerably flattened, the first segment only a little more than four times as long as its greatest width near the distal end Middle and hind coxæ, trochanters, femora and tibiæ black Basal two-thirds of the first, the basal half of the second and the base of the third tarsal segments of the middle leg greyish yellow the rest of the middle tarsus nearly black Basal two-thirds of the first and the basal half of the second hind tarsal segments diffusedly yellow, the rest of the tarsi black The hind basitarsus not conspicuously enlarged (Plate XVI, fig 11), its length being 0 7 of that of the hind tibiæ and its greatest breadth about 0 6 of that of the latter and only about one-fourth its own length It is nearly parallel-sided Fore femora and the pale parts of legs with fine golden pubescence

Wing length and halteres as in female

PUPA

Size about 2 5 × 0 9 mm

The integument of the head and thorax brown, practically free from disc-like tubercles, a few comparatively minute ones being present scattered about on the posterior one-third or so of mesonotum The head and thorax bear the usual number of trichomes which are moderately long and simple In one specimen instead of four pairs of trichomes on the dorsal surface of the thorax, there are five pairs, a comparatively slender pair being placed a little behind the two sub-median pairs Hooks and rows of cuticular spines on the abdomen are as in *himalayense* (Puri, 1932) except that there is no strongly chitinized sensory hair or spine on the ventral surface of segment 4 and there is no row of spines on the dorsal surface of segment 9 The pair of subterminal spines is absent

Respiratory filaments (Plate XVI, fig 12) about 1 2 mm long (nearly half the length of the pupa), light grey, 6 on each side, arranged in three pairs, very much resembling those of *rufibasis* (Puri, 1932*a*) The upper and the lower pairs of filaments have a short stalk while the middle one arises directly from what appears as the stalk of the upper pair The uppermost filament is directed upwards and a little forwards and after a short course bends downwards more or less at right

this species has not been recorded so far from India, at least in the Oriental region Cannanore from where the two specimens of *S ornatum* are supposed to have been collected, is situated on the Malabar Coast (South India) and lies in the Ceylonese sub-region, having a hot and humid climate, quite different from that found anywhere in the Palæarctic region

Considering all this it seems probable that the two specimens have somehow been wrongly labelled and were originally collected from some locality in Europe and were sent to the Museum at Pusa from Cannanore

Summary

1 Male and female of *S howletti* sp n, one of the largest Indian species, have been described in detail

2 Male, female and pupa of *S hirtipannus* sp n, one of the smallest Indian species, closely resembling *S nitidithorax* Puri and *S irridescens* de Meijere, have been described

3 Both the species belong to the subgenus *Simulium* with the basal section of radius bare, fore tarsi with a large silvery white spot on the outside, claws of female with a small sub-basal tooth and tergites of abdominal segments 6–8 (in female) shining

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Puri, I M (1932)	Studies on Indian Simuliidæ, Part I	*Ind Jour Med Res*, **19**, No 3, pp 883-898
Idem (1932*a*)	Studies on Indian Simuliidæ, Part II	*Ibid*, **19**, No 3, pp 899-915
Idem (1932*b*)	Studies on Indian Simuliidæ, Part III	*Ibid*, **19**, No 4, pp 1125-1143

Note.—IN PARTS I TO III OF THIS STUDY, WHEREVER THE 8TH ABDOMINAL STERNITE OF THE FEMALE HAS BEEN DESCRIBED, THE ANTERIOR BORDER HAS WRONGLY BEEN MENTIONED AS THE POSTERIOR AND VICE VERSA

Explanation of Plate XV

Simulium howletti sp n

Fig 1 Dorsal surface of the head and thorax of the type female showing the mesothoracic ornamentation, as seen from in front

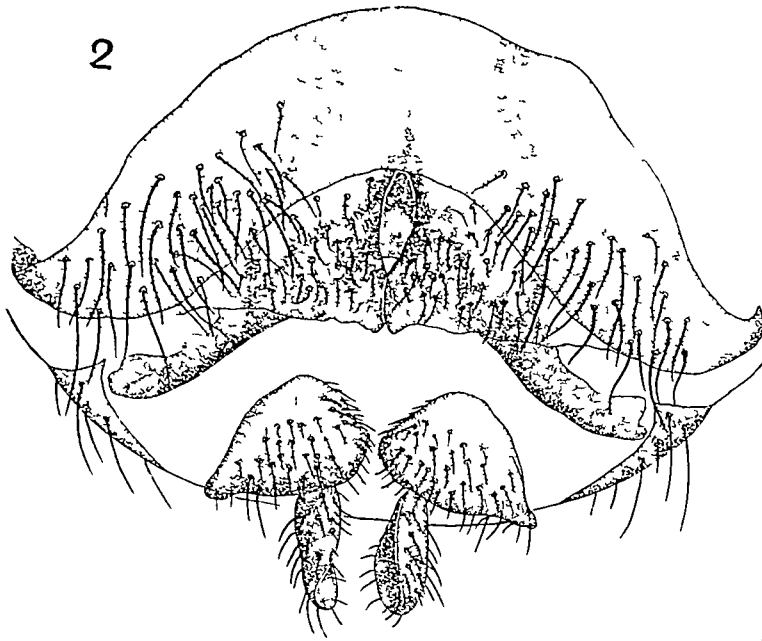
„ 2 Ventral view of terminalia of a paratype female

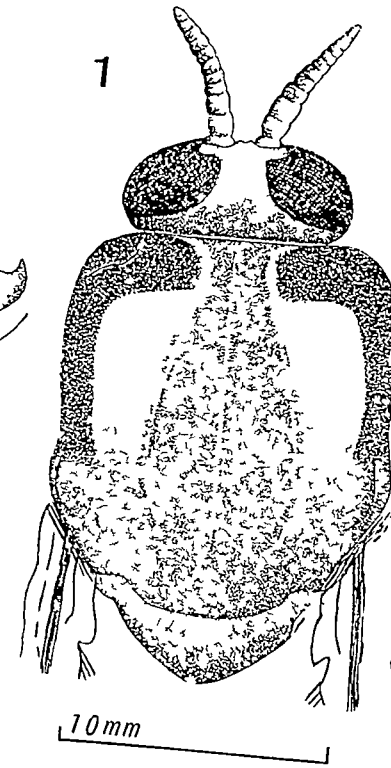
„ 3 Ventral view of genital armature of the type male specimen Left style not shown

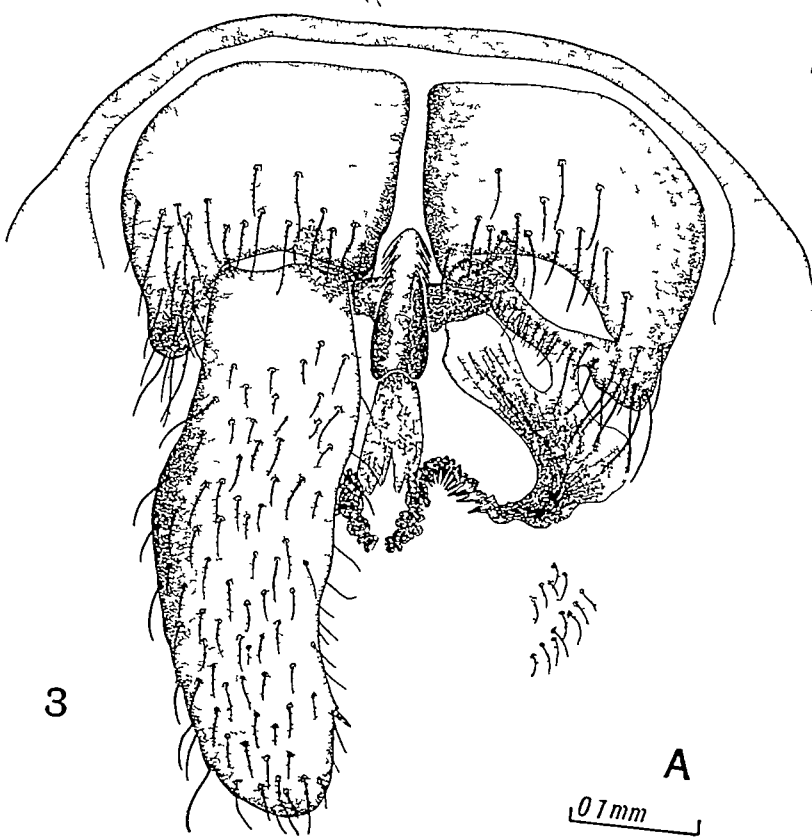
„ 4. Externo-ventral view of the left style

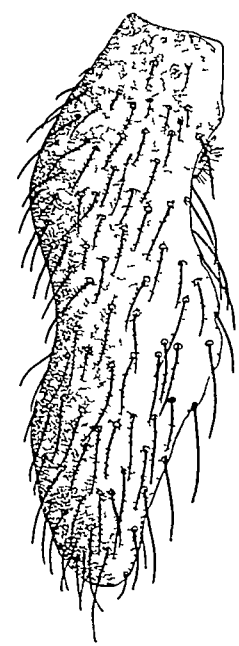
(A —Scale for Figures 2, 3 and 4)

PLATE XV









I M PURI *del*

Ind Jour Med Res, XX, 2, October, 1932

STUDIES ON INDIAN SIMULIIDÆ

Part V.

SPECIES AND VARIETIES OF THE *STRIATUM* SERIES

BY

I M PURI, M SC (Punjab), PH D (Cantab), F E S,

In-Charge, Inquiry on the Indian Simuliidæ, Culicoides and other Blood-Sucking Midges

(*From the Central Research Institute, Kasauli*)

[Received for publication, July 11, 1932]

THE species and varieties dealt with in this paper belong to the subgenus *Simulium*, having fore tarsi flattened and the tergites of abdominal segments 6–8 shining They are all characterized by having striped thorax and simple claws in the female and 10 respiratory filaments in the pupal stage, differing from all the other species described so far, in Parts I to IV of this study, in having an inconspicuous dull grey spot on the outer surface of fore tibiæ instead of the bright silvery white spot present in the latter species

A number of *striatum*-like species have already been described from the Oriental region Brunetti (1911) has described *S grisescens* from a single male specimen collected at Kurseong (Darjeeling District, Eastern Himalayas), 10–26 IX 1909, and *S striatum* (Brunetti, 1912) female from 5 specimens collected at Peradeniya, Ceylon (VI 1910–VII 1911), two species *S eximium* and *S argyrocinctum* (males and females) have been described by de Meijere (1913) from Dutch East Indies and Senior-White (1921) has described *S latistriatum* female from two specimens collected at Coonoor (Nilgiri Hills, S India), September 1920 Senior-White has also described a male as of *S striatum* Brunetti, from 8 males and 3 females all collected at Suduganga, Metale, Ceylon (XII 1919–III 1920), but Dr F W Edwards, after examining these specimens, which are now in the British Museum, has written to me to say that all Senior-White's specimens (males and females) which he had identified as *S striatum* Brunetti belong to the *atratum* group

During the last five years or so I have had occasion to collect simulids from various parts of India and have bred nearly 900 specimens (males and females) out of isolated pupæ belonging to *striatum*-like species A detailed examination of these specimens has shown that they belong to six, somewhat distinct, forms, which are very closely allied to one another, the male as well as the female terminalia of all these being more or less identical As these forms show a certain amount of variation in the colour of their legs and in the presence or absence of hairs on the basal section of the radius in the female, it is rather difficult to decide definitely as to which of them corresponds to the various species, belonging to the *striatum* series, already known from the Oriental region, particularly because of the some what incomplete description of those already known Dr Edwards has very kindly sent me male specimens of *S eximium* de Meijere and *argyrocinctum* de Meijere along with the pupa of the former and a paratype female of *latistriatum* Senior-White A study of these specimens has convinced me that *S eximium* de Meijere has not been collected so far from India, while *S argyrocinctum* which according to Dr Edwards has also 10 respiratory filaments in the pupal stage may be a synonym of *S grisescens* Brunetti, though the mesothoracic pubescence in the male specimen, sent by Dr Edwards, is decidedly coarser than in the latter species After a careful examination of the collection at my disposal, including the type female of *S striatum* Brunetti, I have come to the conclusion that *S striatum* and *latistriatum* are synonyms The type female of *striatum* Brunetti, which is not in a good condition, has decidedly darker legs than in the latter species, but the large number (about 220) of specimens of this species, collected from various parts of South India, do show a certain amount of variation in the colour of their legs, which variations are covered by Brunetti's original description for *striatum* female *S grisescens* Brunetti appears to occur practically all over India and is comparatively a much darker species than *striatum* Brunetti (=*latistriatum* Senior-White)

Simulium (Simulium) striatum Brunetti (1912)

= *Simulium latistriatum* Senior-White

FEMALE

Head dark grey, with short black hairs on the occiput, a few present also on the face and along the lateral borders of the frons Frons greyish black, shining, not very broad, its greatest width near the top is only a little more than half its length, somewhat narrowed in the region of the antennæ Face dusted with ash grey* *Antennæ* In the type female the scape and two basal flagellar segments

* Face is not of the same colour as the frons, as erroneously described by Brunetti

orange-yellow, the rest brownish with very fine pale pubescence In certain lights the whole appearing orange-yellow The colour of the antennæ, however, does not appear to be constant, that of the scape and one or two basal flagellar segments varies from yellow to orange, while that of the rest from orange to reddish brown or nearly black Palpi nearly black, the various segments of usual proportions

Thorax—Mesonotum covered with a fine golden pubescence When viewed from in front the mesonotum is ash grey with a median and two sub-median black stripes forming the same type of lyre-shaped pattern as in the *lineatum* series The two sub-median lines are continued along the anterior border and are connected to broad stripes running along the lateral border The width of the median and the two sub-median stripes are somewhat variable but in the majority of specimens they are all of about the same width, while the outermost stripes running along the lateral borders are nearly twice or more than twice as wide as the former The inner edge of the outermost stripe is produced slightly inwards a little behind its anterior end* All the five black stripes are connected to one another practically in level with the wing bases The colours are as usual reversed when the mesonotum is viewed from behind Scutellum dark brown, covered with a golden pubescence and having long black marginal hairs Pleuræ slate coloured, with ash grey reflections, membranous area bare

Abdomen—The first segment and the basal scale brownish black, the fringe of long hairs dark, somewhat golden, segments 2–5 also brownish black, the second with light grey dusting on dorsum as usual Tergites of segments 6–8 shining black, with a few dark hairs scattered on them Venter somewhat brownish *Terminalia* (Plate XVII, fig 1) A pair of small, lightly chitinized, more or less semi-circular plates present on the ventral surface of segment 7 Hairs (macrosetæ) on the ventral surface of this segment short and simple, somewhat scattered though those on the semi-circular chitinizations uniformly arranged and placed comparatively closer than on the rest of the segment (Plate XVII, fig 2) Sternite of segment 8 moderately wide, anterior edge broadly curved, posterior with a deep somewhat rectangular notch in the middle, fairly strong macrosetæ on the postero-lateral portions, those on each side of the notch very short, the region anterior to the notch bare Anterior gonopophyses of moderate size, placed in the notch, bearing a large number of macrosetæ, posterior region with microsetæ only, interno-lateral border not strongly chitinized, posterior rounded Paraprocts rather large, moderately chitinized, cerci comparatively small

Legs—Fore coxæ yellow, posterior ones brownish black All trochanters yellow, the posterior ones may be somewhat brownish yellow In the various

* It is present even in the paratype specimen of *latistriatum* sent by Dr Edwards

specimens the femora, especially of the posterior pairs of legs, vary in colour from yellow to brownish yellow* , when the latter, they are brown towards the distal end in some specimens Fore tibiæ yellowish brown at the base, gradually changing to dark brown on the distal half to two-thirds, the outer surface with inconspicuous grey dusting , tarsi black, moderately flattened, the first segment a little less than 5 times its greatest width near the distal end Segments one and three with the usual pair of long black hairs sub-terminally on their posterior border Middle tibiæ yellow to brownish yellow on the basal half, gradually becoming brown to dark brown distally, proportions variable in different specimens†, posterior surface with a pale white sheen basally , first tarsal segment pale yellow with a black tip, second and third segments pale yellow basally the rest of tarsi black, the amount of yellow on the various tarsal segments variable in the different specimens Basal one-third to two-thirds (usually the basal half) of hind tibiæ yellow, gradually becoming dark brown to black distally, posterior surface with a pale whitish sheen basally, basal two-thirds of the first and half of the second tarsal segments pale yellow, the rest of hind tarsi black Pedisulcus well marked , calcipala of moderate size, extending up to the former All claws simple, without a sub-basal tooth All yellow parts of legs with fine golden pubescence

Wings —Normal, hyaline , radius, as a rule, bare up to the fork (out of 105 female specimens examined only four showed 2 to 4 black hairs proximal to the fork) , radial sector a simple concave vein *Wing length* Specimens from cold places (South Indian Hills) particularly those collected during the winter months are comparatively larger than those from the plains, the average wing-length of twelve specimens from Coonoor, Kodikanal and Peermade is 2 65 mm , while of ten specimens, out of those collected from Savantvadi Ghat Road and Frazerpet (Mercara) it is only 2 18 mm The wing-length in the paratype specimen of *latistriatum*, collected at Coonoor in September is 2 17 mm , while that in the type of *striatum* from Ceylon it is only about 2 0 mm Halteres pale lemon yellow

Posterior end of buccal cavity with a few rounded protuberances on the ventral surface, the posterior border slightly thickened into a rim and produced into the pharyngeal cavity as a small rounded projection with more or less smooth walls Furca and spermatheca of the usual form

MALE

Head black, with a fringe of short black hairs on the occiput Face dusted with ash grey, with sparse black hairs along its borders Antennæ nearly black,

* All femora brownish yellow in the paratype *latistriatum* Senior White sent to me by Dr Edwards

† In the paratype specimen of *latistriatum* the middle tibiæ are brownish yellow throughout

but in a number of paratype specimens, the scape and basal half of one or two basal flagellar segments reddish brown, the rest nearly black, the whole with very fine pale pubescence Palpi black

Thorax —Mesonotum velvet black covered uniformly with fine copper-coloured pubescence, that along the lateral borders golden and comparatively coarser than that on the top In the fore corners are a pair of large shimmering silvery white spots, narrowly or broadly separated in the middle line, about half of those spots, only reflects light at one time In certain lights the mesonotum shows a broad ash grey border laterally and light grey posteriorly Scutellum velvet black anteriorly, covered with rather coarse copper-coloured pubescence and has a fringe of long black hairs Pleuræ slate grey, membranous area bare

Abdomen velvet black, with sparse fine coppery pubescence dorsally and the usual silvery spots on segments 2 and 5–7 Long hairs on the basal scale somewhat golden black *Genital armature* (Plate XVII, fig 3) The coxites are short, much broader than they are long and bear a comparatively large number of macrosetæ scattered on them The styles are comparatively small They gradually narrow down behind their widest portion near the base and are of uniform width in the distal half Their length is a little more than three times their greatest width near the base and about five times their width about the middle From their dorso-internal surface each sends a small triangular process directed inwards and a little forwards, bearing small teeth on its anterior free surface Each style bears a single short sub-terminal spine on its inner edge The inter-coxal piece (Plate XVII, fig 4) is somewhat saddle-shaped It has a rather broad base which is continued downwards and a little forwards as a short process narrowing down suddenly to a more or less rounded distal end This process may be flattened, tongue-like or it may be cone-shaped distally, depending upon its being collapsed or distended respectively The ventral surface of the inter-coxal piece bears a large number of minute setæ arranged in irregular rows The spines forming the groups on each side of the genital opening are comparatively large

Legs —Fore coxæ, trochanters and femora greyish yellow, the last somewhat dark grey near the distal end , tibiæ nearly black with yellowish black bases, the outer surface with a slight pale greyish sheen , tarsi black, moderately expanded, segment 1 a little more than five times its greatest width near its distal end Middle coxæ black , trochanters and femora greyish yellow, dark grey near the tip , tibiæ yellowish grey on the basal one-fourth to one-third, gradually becoming brownish black distally, the posterior surface with a pale ash grey sheen basally , basal half of first tarsal segment brownish yellow, the rest of tarsi nearly black Hind coxæ black , trochanters yellowish grey , femora yellowish grey on the basal one-third gradually becoming darker distally to dark grey (nearly black) on the distal one-third , tibiæ black, with a somewhat yellowish base , the basal half of the first and

second tarsal segments pale yellow, the rest of hind tarsi black The hind basi tarsus (Plate XVII, fig 5) is moderately enlarged It is about three-fourths the length of the hind tibiæ (0 75 of the length of the latter) and proportionately of about the same width, its greatest width about its middle being 0 27 of its length (while the greatest width of the tibiæ is 0 26 of its own length) The yellowish parts of the legs bear golden pubescence

Wings—As in female, radius always bare up to the fork Wing length in the type specimen from Coonoor is 2 6 mm , the average length varying as in the female Halteres pale yellow varying in colour in the various paratype specimens from yellow to orange yellow

Male described from 15 paratype specimens bred along with a number of females, out of isolated pupæ, collected from Coonoor (26–31 xii 1927) About 100 males and 100 females of this species also bred out of isolated pupæ from various places in South India Type male in my own collection

PUPA

Size of pupæ from Coonoor and Kodikanal about 2 8 × 1 0 mm , while of those from warmer localities it is comparatively smaller

The integument of the head and thorax brownish, covered with comparatively large disc-like tubercles The head bears the usual three pairs of trichomes any of which may be bifid Unlike most of the other species dealt with already in Parts I to IV of this study, the thorax bears 5 instead of 4 pairs of trichomes which are moderately long and split into 2–4 branches a little above their base The cuticular hooks on the dorsal as well as the ventral surface of the abdomen and the pair of sub-terminal spines are as in *himalayense* (Puri, 1932), except that there is no strongly chitinized sensory hair or hook on the ventral surface of segment 4 Dorsally there is a row (broken up in the middle) of backwardly directed short cuticular spines on segment 8 only, a few may, however, be present also on segment 9

Respiratory filaments (Plate XVII, fig 6) are a little less than half the length of the pupa, their length being about 1 2 mm , 10 on each side, the upper five much shorter than the lower There is an upper pair with a short stalk, ventral to which are two sets of three filaments, an inner and an outer set, and below these another pair with a very short stalk Each of the sets of three is composed of a stalked pair with a third filament arising ventrally, practically from the base of the stalk All the filaments spread out somewhat fan-like, the uppermost and the lowermost filaments running practically in line with each other, the former directed upwards while the latter downwards The surface of the filaments is raised into ridges which form a recticular pattern, the ridges covered with large tubercles while the interspaces with very minute ones

Cocoon (Plate XVII, fig 7) from Coonoor about 3 8 × 1 3 mm (that in pupæ from warmer places comparatively smaller), not covering the pupa completely, its length up to the dorsal end of the opening being only about 2 3 mm It is brown to dark brown in colour, boot-shaped, its opening directed upwards and a little forwards It is loosely woven, comparatively more so near the anterior end where the web shows a pretty pattern with large interspaces between the strands

DISTRIBUTION

The female of this species was originally described from specimens collected at Peradeniya, Ceylon, and I have bred out large numbers of specimens, both males and females, belonging to it from pupæ collected from various streams in Coonoor (6,000 ft), Nilgiri Hills, South India, 26–31 xii 1927 (type male) , from small streams crossing the Ghat Road between Belgaum and Savantvadi (Bombay Presidency), 29 xii 1930 , a few from below Sivasamudram Falls, Cauvery River, Mysore State, 6 i 1931 , large numbers from the rushing water of the Cauvery River near Frazerpet (Coorg), 9 i 1931 , from River Sampaj and various small streams crossing the Mangalore-Mercara Road (Coorg), 10 i 1931 , from small streams on Munjamalay T E , Vandiperiyar (Peermade Hills), 17 i 1931 , from various small streams in Peermade (Peermade Hills, Travancore State), 17–18 i 1931 , from above Kottalam Falls, Tinnevelly District (Madras Presidency), 20 i 31 , from streams in Kodikanal (7,000 ft), Palni Hills (South India), 24–25 i 1931

S latistriatum Senior-White female, described from Coonoor, appears to be a synonym of *S striatum* Brunetti and the male specimen described by Senior-White from Suduganga, Ceylon, as that of *striatum* does not belong to this species but to some species belonging to the *atratum* group—to which *S pattoni* Senior-White belongs

Simulium (Simulium) lineothorax SP N

This species closely resembles *S striatum* Brunetti, differing from it mainly in the colour of the legs of both males and females and in the male having a striated thorax like that in the female

FEMALE

Head, thorax and *abdomen* as in *S striatum*, except that the scape and two basal flagellar segments of the antennæ are dark brown and the rest practically black, the whole with a pale pubescence which in certain lights appears somewhat golden In the type specimen the black and grey lines on the mesothorax are all practically of the same width, the black lines being comparatively broad and the

grey ones narrow but in the various paratype specimens the relative width of these lines is very variable The slight inward extension of the inner border of the outermost black stripe, a little behind the anterior end, is not as well marked as in *striatum*

Legs —Fore coxæ and trochanters pale yellow, femora brownish yellow near the base, gradually becoming dark towards its distal end, tibiæ nearly black, slightly yellowish near the base, the outer surface with some inconspicuous pale grey dusting, tarsi black, flattened, the first segment a little more than four times as long as its greatest width near the distal end Middle and hind coxæ black, trochanters brownish yellow Middle femora nearly black, slightly yellowish basally, in some paratype specimens they are brownish throughout, tibiæ somewhat yellowish on the basal portion gradually becoming dark grey to nearly black on the distal three-fourths, the posterior surface with a whitish sheen basally, first tarsal segment pale yellow with a yellowish black tip, second yellowish basally, the rest of tarsi black Hind femora and tibiæ black, with pale yellowish bases, the posterior surface of the latter with some whitish sheen basally, basal two-thirds of the first and basal half of the second tarsal segment pale yellow, the rest of hind tarsi black All claws simple All legs with sparse, fine, golden pubescence

Wings —Normal, hyaline, radius bare up to the fork* Wing length about 2 7 mm Halteres pale lemon yellow

MALE

Head as in *striatum* male The antennæ with the scape and the two basal flagellar segments somewhat reddish

Thorax —Mesonotum sparsely covered with fine golden pubescence When viewed from in front it is ash grey with black longitudinal stripes arranged the same way as in the female but are comparatively narrower In most of the paratype males the sub-median black stripes, as in the female, are continued along the anterior border as a broad band connected to the stripe running along the lateral border, but in the type specimen the sub-median stripes are not connected to the broad band anteriorly As the specimen is turned forwards the black anterior band in each fore corner changes to a large silvery white spot Colours of the various stripes are as usual reversed when viewed from behind Scutellum brownish black, covered with rather coarse copper-coloured pubescence and has a fringe of long black hairs Pleuræ slate grey, membranous area bare

Abdomen (genital armature, etc) as in ♂ *striatum*

* In one out of seven of the paratype specimens there are two hairs even on the portion of radius proximal to the fork

Legs —Fore coxæ, trochanters and femora greyish yellow, the last somewhat dark grey distally, tibiæ practically black with a somewhat yellowish black base, the outer surface with a slight pale grey sheen, tarsi black, moderately expanded, first segment about five times as long as its greatest width near its distal end Middle and hind coxæ black, trochanters, femora* and tibiæ also black with pale yellow bases, basal half of the first and the base of the second tarsal segments pale yellow, the rest of tarsi black The hind basitarsus as in *S striatum*

Wing as in *striatum* Wing length about 2 5 mm

PUPA

(Respiratory filaments as well as cocoon) as in *striatum*

Described from 8 females and 10 males all bred out of isolated pupæ collected from a large hill stream about 2 miles below Haflong (Naga Hills, Cachar, Assam), 1 ii 32 A single damaged male specimen of this variety was also bred out of a pupa collected from a large stream near Mettupalayam at the foot of Nilgiri Hills, South India (8 i 28)

Types in my own collection

Simulium (Simulium) grisescens Brunetti (1911)

The male of this species was described by Brunetti (1911) from a single specimen collected at Kurseong (10–26 ix 1909) by Mr Lynch I have since bred out a large number of specimens both males and females belonging to this species, from pupæ collected from various parts of India As male genital armatures are similar in practically all the closely allied forms and thus of no help in differentiating this species from others in this group, it would not have been possible ordinarily to fix the identity of the species but the dark colour of the legs and the size of the hind basitarsus as found in the type male specimen are enough to identify it from all other forms found in Northern India Consequently the specimens, both males and females dealt with below, undoubtedly belong to *S grisescens* Brunetti

This species closely resembles *S striatum* from which it differs mainly in the colour of the legs in both the sexes, those of *S grisescens* being much darker than in the latter species

FEMALE

Head, thorax and *abdomen* as in *S striatum* Brunetti In the type specimen the scape and two basal flagellar segments of the antenna are brownish yellow, the rest nearly black, the whole with a fine pale pubescence The colour of the

* In some paratype specimens the middle femora are comparatively paler than the hind pair

18 i 31 There are two females specimens belonging to this species in the Indian Museum, collected by Dr S Kemp, one marked 'Rotung, Abor Expedition, 21 xii 11 (Bites) and the other 'Upper Rotung, Abor Expedition, 6 i 12' In the collection of the Imperial Agriculture Institute, Pusa, is a single female of this species marked 'Lower Ging, Lebong, 4,500 ft 2-9 vi 1909, F M H'

Simulium (Simulium) grisescens VAR **palmatum** NOV VAR

The adults, both male and female of this variety, are identical with those of the type species in every way but the differences in the pupal stages of the two forms are so marked and constant that I have no hesitation in describing this form as a distinct variety The pupæ of this variety have so far been collected only from parts of peninsular India, very often occurring together with those of *striatum*, *gurneyæ*, *pattoni* and *aureohirtum* but extremely rarely with those of the type species

PUPA

Size about 2 5 × 1 0 mm

Integument of the head and thorax varies from light brown to dark brown, somewhat shining, being almost bare, the disc-like tubercles absent on the head and on the anterior three-fourths of the thorax, a few minute ones present on the posterior one-fourth and an irregular row of them also along the mid-dorsal line Thoracic trichomes moderately long, usually simple, may be split into two in some specimens Cuticular hooks, rows of spines and the sub-terminal spines as in *striatum*

Respiratory filaments (Plate XVII, fig 8) are comparatively short, being only about 1 0 mm , ten in number, arranged more or less the same way as in *striatum*, but the upper five filaments are only half to one-third the length of the lower five and are much dilated in their basal half, spreading out from the main stalk like the five fingers of the hand The walls of the filaments are comparatively much thinner and the portions of the upper five filaments beyond the dilated region, do not stand out rigid but are usually collapsed and often broken off, so that at first sight the upper five filaments appear only finger-like without any filament-like distal portion The surface of the filaments is covered with minute tubercles which are more or less uniformly distributed (without any ridges) those on the dilated portions being much more widely separated from one another

Cocoon is about 3 2×1 2 mm not covering the pupa completely Golden brown in colour, boot-shaped, as in the type species It is closely woven, smooth and shining, without any interspaces or windows in the web The anterior end is also without any spaces and has a fairly strong more or less uniform rim, without any ornamentation

Described from 130 specimens males and females all bred out of isolated pupæ collected from various places in Southern India Types (from Savantvadi, 29 xii 30) in my own collection

DISTRIBUTION

Pupæ of this variety have been collected in large numbers from the following places From streams crossing the Ghat Road between Belgaum and Savantvadi (Savantvadi State, Bombay), 29 xii 30, on rushes in the fast water of the Cauvery River near Frazerpet (Coorg), 9 i 31, from streams crossing the Mangalore-Mercara Road (10 to 14 miles below Mercara), 10 i 31, streams in Peermade (Peermade Hills, Travancore), 17-18 i 31, and only two specimens each from Pudapadi (Calicut, Malabar), 14 i 31, and Vandiperiyar (Peermade Hills), 17 i 31

Simulium (Simulium) consimilis SP N

This species closely resembles *S striatum*, the females differing mainly in the colour of the legs and the male in the relative size of the basitarsus

FEMALE

Head, *thorax* and *abdomen* as in *striatum* In the type female the scape and two basal flagellar segments are golden yellow, while the rest of the antennæ are greyish brown The colour of the antennæ is, however, variable, that of the scape and two basal flagellar segments varying from yellow to reddish brown and that of the rest from pale brown to dark brown or nearly black

Legs —Fore coxæ pale yellow, posterior ones nearly black, all trochanters yellow Fore femora brownish yellow, somewhat darker near their distal end, tibiæ nearly black, slightly yellowish brown basally, the outer surface with some inconspicuous pale greyish dusting, tarsi black, moderately flattened, the first segment about five times as long as its greatest width near the distal end Middle femora and tibiæ brownish yellow*, the latter somewhat darker (dark brown) on the distal half, the posterior surface with a whitish sheen basally, first tarsal segment pale yellow with a dark brown tip, second and third dark brown, the former yellow on the basal half, the end tarsi black Hind femora yellowish brown basally, gradually becoming dark brown to nearly black towards their distal end, tibiæ nearly black, the basal one-fourth pale yellow, the posterior surface with some whitish sheen basally Basal two-thirds of the first and basal half of the second tarsal segment pale yellow, the rest of the hind tarsi black All claws simple All legs with sparse fine golden pubescence

* In some specimens they are somewhat darker and the femora may be brown throughout

Wings.—Normal, hyaline, radius in the type female bare up to the fork but in a number of paratype and other specimens a few hairs are present on the portion proximal to the fork. Wing length about 2·57 mm. Halteres lemon yellow.

MALE

Head, thorax and *abdomen** as in *striatum*, the antennæ, however, are dark brown with a black tip, the colour varying in the paratype and other specimens from yellowish brown to reddish brown. In some specimens the antennæ are yellowish brown throughout their length. The mesothoracic pubescence is golden and comparatively coarser than in *striatum*.

Legs.—Fore coxæ, trochanters and femora yellow, the last somewhat greyish yellow near the distal end, tibiæ nearly black, the outer surface with a faint pale greyish sheen, tarsi black, moderately flattened, the first segment about five times as long as its greatest breadth near its distal end. Middle coxæ brownish black, trochanters and femora† greyish yellow, the latter dark grey near the distal end, tibiæ greyish yellow on the basal third or so, gradually becoming dark brown distally, the posterior surface with an ash grey sheen basally, first tarsal segment pale yellow with a black tip, second pale yellow on basal half, the rest of tarsi black. Hind coxæ brownish black, trochanters pale yellow, femora brownish black with a pale yellow base, tibiæ nearly black, the posterior surface with a whitish sheen basally, a little more than the basal half of the first and the basal half of the second tarsal segment pale yellow, the rest of hind tarsi black. The basitarsus of the hind leg is nearly parallel-sided, very little enlarged (Plate XVII, fig. 9). It is about three-fourths the length of the hind tibiæ (0·74 of the length of the latter), but its greatest width about its middle is only about two-thirds that of the latter, nearly one-fifth its own length (being 0·21 of its length, while that of the latter being 0·26 of its own length). The yellowish parts of the legs with fine golden pubescence.

Wing as in *striatum*. Wing length 2·4 mm. Halteres yellow.

PUPA as in *grisescens*.

Described from 34 males and 36 females all in good condition and bred out of isolated pupæ.

Types and paratypes (from Chandigarh, Ambala District, Punjab) in my own collection.

DISTRIBUTION

Specimens, both males and females, of this species have been bred out of isolated pupæ so far collected from the following places. A mill stream near

* In some specimens the tip of the inter-coxal piece appears somewhat pointed but this does not seem to be constant.

† In some paratype specimens the femora and tibiæ are comparatively darker both appearing dark brown, the former a little lighter than the latter.

Chandigarh Dâk Bangalow (Ambala District), 26 x 26 (types), from River Kuthar (October 1927) and Nahan stream (August 1929) both near Kasauli, from River Ghagar near Pinjaur (Patiala State), 6 iv 30, from a stream south of Chhota Simla, 5 x 30, and a single specimen from River Leh, near Sohan Bridge, Rawalpindi, 10 ix 30

Simulium (Simulium) pallidum SP N

FEMALE

Head grey with slender somewhat pale golden hairs on the occiput also present on the face and a few along the lateral border of the frons Frons dull grey (not shining as in the other species of this group), not very broad, its greatest width at the top is about half its length, somewhat narrowed in the region of the antennæ Face dusted with ash grey Antennæ reddish dark brown, three basal segments somewhat paler The colour of the antennæ varies in the various paratype specimens from yellowish brown to reddish brown or dark brown Palpi brownish black, the first two segments somewhat yellowish brown, the various segments of the usual proportions

Thorax —Mesonotum covered with fine pale golden pubescence When viewed from in front the mesonotum appears dull pale grey with a narrow median and two somewhat broader more or less greyish black stripes, forming the same type of pattern as in *striatum* The two sub-median black stripes are continued along the anterior border and are connected to a narrow dark stripe running along the lateral border Unlike the stripe along the lateral border in the other species of the *striatum* group that in this one is narrow and inconspicuous So that the sub-lateral grey stripes are much broader than the sub-median pair All the greyish black stripes are connected to each other in the region of the wing bases The colours are as usual reversed when the mesonotum is viewed from behind Scutellum dark brown, covered with a golden pubescence and having long pale golden marginal hairs Pleuræ with dark grey reflections, membranous area bare

Abdomen —Closely resembles that of *S striatum* The colour in the type female is comparatively lighter but varies in the different paratype specimens The *terminalia* are as in *striatum* except that the thinly chitinized semi-circular plates as found on the ventral surface of segment 7 are absent and the macrosetæ on this segment are somewhat uniformly and more or less widely scattered Moreover, the macrosetæ on sternite of segment 8 are comparatively fewer and shorter

Legs —Fore coxæ brownish yellow*, posterior ones nearly black All trochanters and femora yellow Fore tibiæ yellow, with the distal one-sixth or so black

* Yellow in many paratype specimens

and the outer surface with a faint greyish sheen, tarsi black, only slightly flattened, the first segment being about six times as long as its greatest width near the distal end Middle tibiæ yellow, the posterior surface with a whitish sheen basally, first and second tarsal segments pale yellow with somewhat brownish distal ends, third pale yellow basally, the rest of tarsi black Hind tibiæ yellow on the basal two-thirds or so gradually becoming nearly black distally, basal three-fourths of the first and basal half of the second tarsal segment pale yellow, the rest of hind tarsi black All claws simple Yellow portions of legs with fine golden pubescence

Wings—Hyaline, radius always bare up to the fork Wing length about 2 6 mm Halteres pale lemon yellow

MALE

Head, thorax and *abdomen* as in *striatum* The pubescence on the thorax and the abdomen, however, is golden and that on the former comparatively coarser

Legs—Fore coxæ yellow, posterior ones black All trochanters and femora yellow Fore tibiæ yellow on basal third, gradually becoming dark brown to nearly black on the distal two-thirds, the outer surface with an inconspicuous pale grey sheen, tarsi black, only slightly flattened, first segment about six times as long as its greatest width near the distal end Middle tibiæ yellow, posterior surface with some whitish sheen basally, first tarsal segment pale yellow with the distal one-fifth nearly black, basal half of second and the base of the third segment yellow, the rest of tarsi black Hind tibiæ yellow on about the basal half, gradually becoming yellowish black to nearly black distally, basal two-thirds of the first and the basal half of the second tarsal segment pale yellow, the rest of hind tarsi black Hind basitarsus very little enlarged, nearly parallel-sided, resembles that of *S consimilis* Its length is about three-fourths that of the hind tibiæ (0 73) but its greatest width is only about two-thirds of that of the latter and one-fifth its own length The yellow parts of the legs with fine golden pubescence

Wing as in the female, length about 2 3 mm Halteres lemon yellow

PUPA as in *grisescens*

Described from 23 males and 54 females all bred out of pupæ*

Types and paratypes (from near Kasauli) in my own collection

DISTRIBUTION

Specimens of this species have been bred out of pupæ so far collected from the following places Small streams crossing the Kasauli-Subathu

*A number bred out of isolated ones

Road, 4,000–5,000 ft, 15 VIII 26 (types and paratypes), River Kuthar (October 1927), Nahan and Karh streams (August 1929), all near Kasauli, from a small stream near Dâk Bungalow, Chandigarh, 26 X 26, River Leh near Sohan Bridge, Rawalpindi, 10 IX 30, and a single female specimen from a stream south of Chhota Simla, 5 X 30

SUMMARY

Six very closely related forms belonging to the group of *striatum*-like species have so far been collected from India They are characterized by having striped thorax and simple claws in the female, 10 respiratory filaments in the pupal stage and by having only an inconspicuous dull grey spot on the outer surface of fore tibiæ (instead of the silvery white spot as found in all those species described in Parts I to IV of this study) The genital armatures of practically all of them are identical, the various forms showing certain constant differences mainly in the colour of their legs

Male and pupa of *S striatum* Brunetti and female and pupa of *S grisescens* Brunetti have been described for the first time, and revised descriptions of the female of the former and the male of the latter have been given

Males, females and pupæ of three new species belonging to this group have been described

A new variety of *S grisescens*, based on the form of the respiratory filaments and certain distinctive characters of the cocoon, has also been described

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Note—IN PARTS I TO III OF THIS STUDY, WHEREVER THE 8TH ABDOMINAL STERNITE OF THE FEMALE HAS BEEN DESCRIBED, THE ANTERIOR BORDER HAS WRONGLY BEEN MENTIONED AS THE POSTERIOR AND VICE VERSA

Explanation of Plate XVII

Simulium striatum Brunetti

Fig 1 Part of ventral view of terminalia of female

„ 2 Part of ventral surface of abdominal segment 7 showing the thinly chitinized semi-circular plate with close lying short macrosetæ (Scale as in Fig 1)

„ 3 Ventral view of genital armature of a paratype male Left style not shown (Scale as in Fig 1.)

„ 4 Ventral view of inter-coxal piece

„ 5 Tibia and tarsus of right leg of a paratype male (Scale A)

„ 6 Part of pupal respiratory filaments of left side (Scale A)

„ 7 Lateral view of cocoon (Scale B)

Simulium grescscens nov var *palmatum*

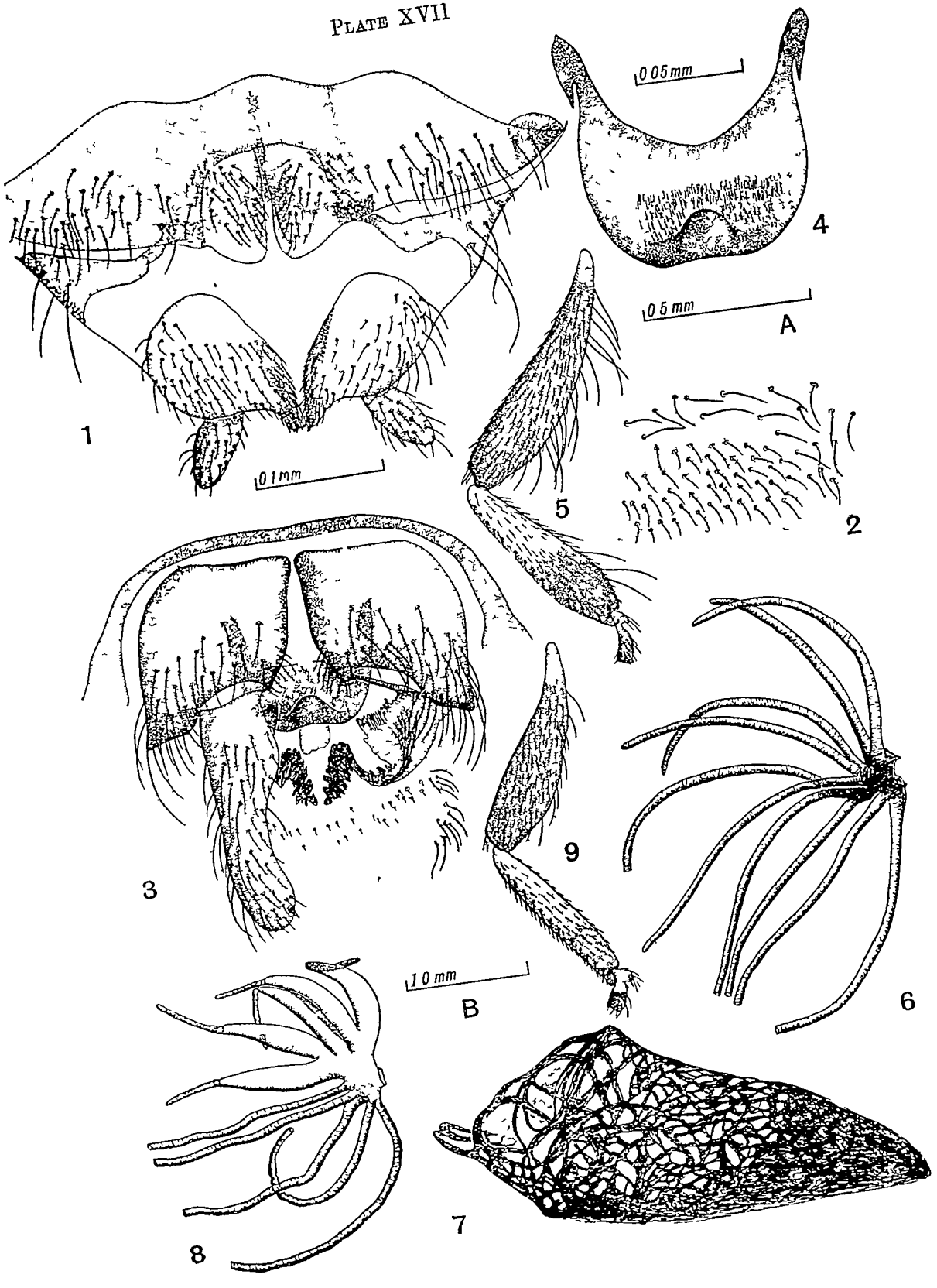
Fig 8 Part of pupal respiratory filaments of left side (Scale as in Fig 6)

Simulium consimilis sp n

Fig 9 Tibia and tarsus of right leg of a paratype male (Scale A)

(A —Scale for Figs 5, 6, 8 and 9 B —Scale for Fig 7)

PLATE XVII



I M PURI *del*

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MORINGA PTERYGOSPERMA (*N O MORINGÆ*)

BY

LIEUT-COLONEL R N CHOPRA, M A, M D (Cantab), I M S,
Professor of Pharmacology, School of Tropical Medicine, Calcutta,

PREMANKUR DE, B Sc, M B (Cal), M R C P (Edin),
Professor of Physiology, Medical College, Calcutta,

AND

NRIPENDRA NATH DE, M B
(*From the Department of Pharmacology, Calcutta School of Tropical Medicine*)

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Moringa pterygosperma Gærtn, also known as *Moringa oleifera* Lam, *H decandra* Willd, and *Guilandina moringa* Linn, is a large and beautiful tree, belonging to the Natural Order Moringæ It is known in Sanskrit as *Murunga, Sing, Shobhanjanavrikshaha*, in Hindi as *Shajnah, Segva*, in Bengali as *Sojna, Sajina*, in Orissa as *Munigha*, in the N-W F P as *Sahajna*, in the Punjab as *Soanjna*, in Bombay as *Sanga* and in Burma as *Dandalonbin* The tree grows wild in the sub-Himalayan tract from the banks of the Chenab to Oudh, and is also commonly cultivated in many parts of India and Burma The leaves, flowers and fruits are all eaten as vegetables The corky, grey bark is about an inch thick and has got longitudinal cracks It yields a coarse fibre which might be utilized in preparing mats, paper or cordage The root is pungent and has the taste of horse-radish, the wood of the root is soft, porous and yellowish The bark of the root is thick, soft and reticulated, it is light brown externally, soft and white internally The gum is opaque and white when it first exudes, but on exposure to air soon changes to pink, dull or mahogany colour on the surface It is insoluble in water and possesses a bland mucilaginous taste, in dry air it is friable, but becomes tough in damp climate due to absorption of nearly 20 per cent of its weight of water

The gum belongs to the tragacanth or hog gum series, but on account of its dark colour has not got much value in European commerce The seeds yield on pressure a clear limpid, almost colourless oil, which saponifies slowly and does not turn rancid on standing It has a specific gravity of 0 912 to 0 915 at 60°F and is almost devoid of odour and flavour It is composed chiefly of olein, margarine and stearine and is considered to be one of the best lubricants for fine machinery by watch makers On account of its property of absorbing and retaining the most fugitive odours, the oil is highly esteemed by perfumers

Medicinal uses – The medicinal virtues of this plant have long been known and appreciated in India It has been frequently mentioned in the Bhavaprakasa and in many other authoritative works on Hindu medicine Almost all parts of the plant e g , root, bark gum, leaf, flower, seed and the seed-oil, have been used for various ailments in indigenous medicine The root made into a poultice or paste is very commonly applied to the skin, either alone or in combination with mustard seeds and green ginger, as a counter-irritant and blistering agent in enlargement of the liver and spleen in children and in painful rheumatic joints The fresh juice of the root is sometimes poured into the ears to relieve otalgia , a decoction of the whole root or the root-bark is used as a gargle for stomatitis and gingivitis The root of the young tree, the root-bark and sometimes the fresh juice expressed from the root are recommended for internal administration for intermittent fever, dyspepsia ascites, enlarged spleen or liver and calculus in the kidney and bladder

The gum is frequently applied externally for the relief of rheumatic joints and to disperse glandular swellings It is also used in some places to produce abortion It would be quite possible to use it as a tent to dilate the os uteri, because it is very tough, and easily swells up in the presence of moisture

The young leaves have been used along with other ingredients in the treatment of dog-bite, scurvy and catarrhal affections of the mucous membrane They are also believed to possess aphrodisiac properties The *hakims* administer the fruit in affections of the liver and spleen, articular pains, tetanus, debility of nerves, paralysis, pustular eruptions, white patches, leprosy, etc

The flowers are considered to possess stimulant and aphrodisiac properties Mohammedan writers describe the flowers as hot and dry and use them as tonics, diuretics and cholagogues The juice has been prescribed with milk as a diuretic, antilithic, digestive and antispasmodic The seeds (*Sweta maricha* or white pepper) are described as acrid and pungent, and are used in the treatment of ascites due to derangement of liver The oil expressed from the seed is used externally for relieving pain of the joints and in gout and acute rheumatism The medicinal virtues of *Moringa pterygosperma* appear to have impressed many early European physicians who visited India, as is evident from the good reports left regarding it, by Taylor, Stewart, Dymock and others

Chemical composition —The chemical examination of the bark was carried out by Dr S Ghosh and Mr Ashutosh Dutt of the Department of Chemistry, and the details will be published by them in due course A short summary of the result is given below —

A preliminary extraction in a Soxhlet's apparatus with different solvents in succession gave the following extracts petroleum ether 0 71 per cent , sulphuric ether 6 47 per cent , chloroform 0 68 per cent and absolute alcohol 2 17 per cent The petroleum ether extract contained some fatty oil, traces of an essential oil and a phytosterol The sulphuric ether extract contained some organic acids and a waxy substance The chloroform extract gave distinct reactions of an alkaloid while the alcoholic extract contained some resins and alkaloids An average of four assays by different methods showed the presence of 0 105 per cent of alkaloids Five hundred and thirty-four grammes of fresh undried bark, when distilled in steam gave only 0 026 g of an essential oil which had a very pungent smell

For the isolation of the alkaloids, the air-dried bark was extracted with rectified spirit, the alcohol was recovered under reduced pressure and the residue taken up with a dilute acid The acid aqueous extract was partially neutralized and allowed to settle, and the clear liquid was filtered and extracted with petroleum ether It was then made alkaline with ammonia and extracted repeatedly with sulphuric ether The ethereal extracts were washed, dried with sodium sulphate and the solvent removed The original alkaline aqueous liquid, which still contained some alkaloids, was then shaken exhaustively with chloroform which removed the rest of the alkaloids The chloroform extract was washed, dried and the solvent removed Both the alkaloidal residues were then converted into hydrochloride and the hydrochloride dried in vacuo The dry residue was then extracted with boiling dry chloroform, which removed the non-crystallizable portion The insoluble residue was recrystallized several times from absolute alcohol until a substance of constant melting point was obtained It is proposed to designate this alkaloid provisionally as *Moringine* The hydrochloride crystallized in white glistening plates melting at 254 4°C and gave crystalline picrate, aurichloride and platinic chloride

The chloroform soluble hydrochloride was purified in different ways and has not as yet been obtained in a crystalline form It is proposed to designate this latter alkaloid as *Moringinine* None of these alkaloids give the chemical and physical tests of ephedrine

Experimental

Our experiments were conducted both with *Moringine* and *Moringinine*, as these appeared to be the chief constituents isolated from the root bark The crystalline alkaloid *Moringine* was found to be comparatively inert The residual

alkaloid *Moringinine* showed physiological activity As this alkaloid could not be isolated in a pure crystalline form, the hydrochloride of the crude base was used in all our experiments The dosage given was therefore somewhat larger than if the alkaloid was in a pure state

Local effects —A 5 per cent solution of the alkaloid applied to the unbroken skin produced no signs of irritation Subcutaneous injection of 1 c c of a 5 per cent solution of the drug in a guinea-pig did not produce any signs of local inflammation The application of a few drops of 1 per cent solution of the drug to the conjunctiva of rabbits had no irritant effect The pupils showed very slight dilatation, but there were no signs of any anæsthesia of the cornea Intravenous injections of 5 to 10 mg of the alkaloid in cats also produced slight dilatation of the pupil

Blood-pressure —Injections of 5 to 10 mg of the alkaloid intravenously in a cat under chloralose or urethane produce a well-marked and persistent rise of blood-pressure varying from 20 to 80 mm of mercury (Graph 3, *e*) The blood-pressure remains high from about 3 to 10 minutes after which it returns to its normal level, but rarely goes down below the normal When the blood-pressure is at its highest, the heart shows a distinct slowing, and the curve sometimes drops a little due to the effect of the high blood-pressure on the vagal centre

The rise of blood-pressure produced may be due to either of the following factors (1) paralysis of the vagal mechanism, (2) stimulation of the sympathetic, (3) direct stimulation of the musculature of the heart, (4) contraction of the blood vessels Further experiments have been performed in order to find out which particular factor is specially responsible for the rise In decerebrated and atropinized animals and in animals treated with large doses of nicotine which paralyse the ganglion cells, the usual rise of blood-pressure is obtained in all these cases after administration of the alkaloid, indicating that neither the vaso-motor centre, the vagal system, nor the ganglia are concerned with the rise The probability therefore is that the rise in pressure is due to a stimulation of the sympathetic nerve-endings or to direct stimulation of the musculature of the heart

The pulmonary blood-pressure is raised and it remains high for some time after the carotid pressure comes down to normal (Graph 1, *d*) This effect is probably brought about by increase in the force of contraction of the heart-beat and accumulation of more blood in the pulmonary circulation, due to constriction of the vessels in other parts of the body, which are more under sympathetic control than the pulmonary vessels

In order to determine whether the drug stimulates the sympathetic nerve endings, repeated small doses of ergotoxin phosphate were injected intravenously till the vaso-motor nerve-endings were paralysed The response obtained as a result of injecting 5 to 10 mg of the alkaloid was recorded before administration of ergotoxin, and this was compared with the response obtained with a similar

dose given afterwards It was observed that an injection of the alkaloid after ergotoxin produced a much smaller rise of pressure, and sometimes no rise whatsoever (Graph 3, *b*) This indicates that the sympathetics are partly, if not wholly, responsible for the action of the drug The small rise of blood-pressure observed after ergotoxin is probably due to direct action of the drug on the musculature of the vessel wall The return of the carotid blood-pressure to the original level without a fall below the normal level, indicates that, unlike adrenalin, the vaso-dilator fibres are not stimulated

Cocainization —To determine if the drug acts on the sympathetics or on the musculature of the vessel wall, we used the cocainization method described by Tainter (1927, 1929) Fifteen milligrams of cocaine per kilogram of body-weight were given subcutaneously to animals anæsthetized with urethane, which also received a subcutaneous dose of 1 mg of atropine per kilogram of body-weight This renders the sympathetic nerves more sensitive, and any stimulation by true sympathetic accelerator drugs produces a greater response *sensitization*—whereas weak direct muscular stimulants show comparatively less response—*desensitization* This experiment was performed on a number of animals and pressor responses obtained with this alkaloid and adrenalin in non-cocainized animals were compared with those obtained in cocainized animals It was observed that the alkaloid from *M pterygosperma* produced a weak response after cocainization, whereas the pressor effect of adrenalin was very much intensified It is reasonable to conclude from this that the alkaloid owes at least a part of its action to stimulation of the smooth muscle of the blood vessels, but it is difficult to say to what extent the resultant action is due to stimulation of the sympathetic nerve-endings or to stimulation of the musculature of the vessel wall The explanation becomes all the more difficult when the stimulant action of the drug on the heart, described later, is also considered Any residual pressor response observed after ergotoxin or cocaine may be due to the action of the drug on the heart or musculature of the vessels

Adrenalectomy —It has been shown by Tainter (1926) that the pressor action of drugs like nicotine, strychnine, etc , in animals depends partly on the integrity of the supra-renal glands, and that the response is different in normal and adrenalectomized animals Experiments with this alkaloid after removal of the adrenals show that its pressor effect is considerably reduced This is probably due to loss of adrenal secretion and consequent vascular and muscular asthenia, and also partly to lack of increased epinephrine secretion which is produced by such drugs

Myocardiograph and cardiometer experiments —Intravenous injections of the alkaloid in animals in which myocardiographic tracings are being recorded show a slight acceleration of the rate of the heart, both the auricles and the ventricles show a definite increase in the force of the beats (Graph 1, *a*) The cardiometer

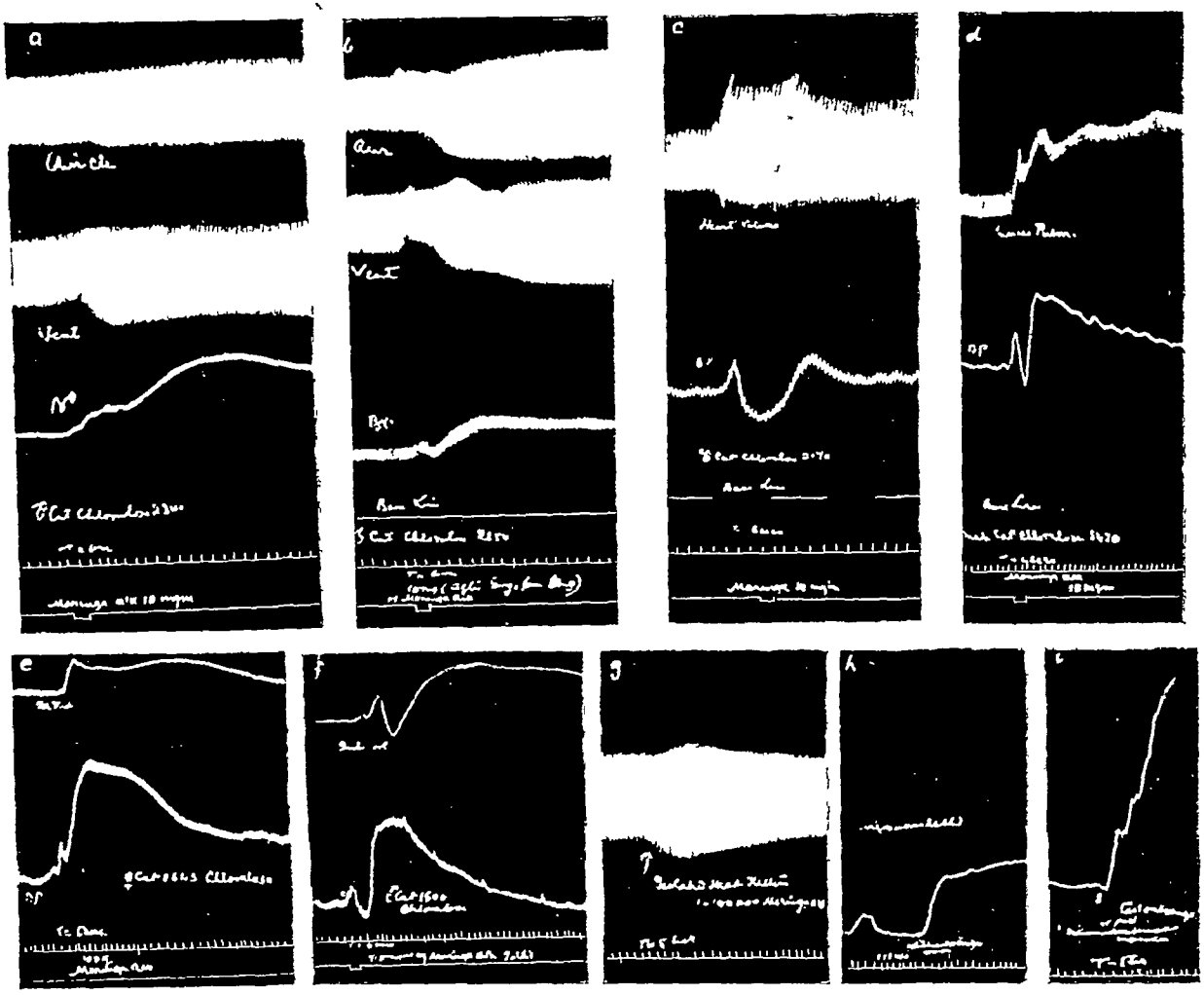
experiments show a marked increase in the volume of the heart (Graph 1, *c*) After large doses however, signs of dilatation are evident, probably due to increased peripheral resistance brought about by constriction of blood vessels Similar results are obtained in a series of experiments in which the vagal endings are paralysed with atropine The effects therefore, are not due to paralysis of vagal endings, but either to stimulation of the sympathetics or direct effect on the cardiac musculature Further, when these experiments have been done on animals, which have received repeated doses of ergotoxin to paralyse the sympathetic endings, injections of the alkaloid show more or less the same effects on the auricles and the ventricles, but a smaller rise of blood-pressure is obtained In fact in most of these experiments the alkaloid produces a greater stimulation of the auricles and the ventricles after administration of ergotoxin (Graph 1, *b*) This fact would suggest that the alkaloid might have some direct stimulant action on the myocardium, though it must be realized that ergotoxin, according to many authorities, does not completely paralyse the sympathetic mechanism of the heart

Isolated heart —Isolated hearts of rabbits and kittens are perfused through the aorta with oxygenated Locke's solution having a pH of 7 2 and temperature of 37 5°C The heart perfused with 1-100 000 solutions of the alkaloid shows a well-marked increase in the amplitude of contraction, the rhythm being only slightly accelerated (Graph 1, *g*) The coronary outflow also shows an appreciable increase All these effects suggest either stimulation of the sympathetic mechanism or direct stimulation of the myocardium The action of the heart after the drug, however, is characteristic and is not like that seen when the inhibitory mechanism is paralysed Moreover, the alkaloid after repeated injections of atropine, still shows increase in amplitude and acceleration of the rhythm This conclusively shows that the action is not on the vagal mechanism After repeated injections of ergotoxin the heart still shows stimulation though to a lesser extent

Perfusion of vessels —Perfusion of blood vessels in the frog, with dilute solutions of the alkaloid by Trendelenberg's method, produce a well-marked constriction Similar results are also obtained when the mesenteric vessels of the cat are perfused with ergotoxin and then with the alkaloid a lesser degree of constriction being observed These facts favour the view that the alkaloid acts mainly by stimulating the sympathetic endings, while the slight constriction observed after ergotoxin is probably due to direct stimulation of the musculature of the vessel wall

Myocardium —An attempt has been made to determine the action of the alkaloid on the myocardium and to see whether it has any depressant action on the heart muscle in large doses, as is the case with alkaloids like ephedrine Small doses such as 1/100 mg and 1/40 mg of this alkaloid have no effect whatever on the myocardiograph tracings of the auricles or the ventricles or on the blood-pressure Doses of 0 5 mg produce a slight rise of blood-pressure and a slight increase in the

Graph 1



(*a*) Male cat, 2 340 g chloralose The tracings from above downwards represent auricular and ventricular movements, carotid blood pressure Upstroke diastole and downstroke systole Ten milligrams of the *Moringa* alkaloid into the femoral vein produce a definite increase of force and slight increase of frequency of the beat and a rise of blood pressure Time=6 seconds

(*b*) Male cat 2 650 g chloralose The tracings from above downwards represent auricular and ventricular movements and blood pressure Ten milligrams of the *Moringa* alkaloid injected after sufficient ergotoxin to paralyse the vaso motor sympathetics show definite stimulation of the auricles and ventricles and slight rise of blood pressure Time=6 seconds

(*c*) Male cat 2 170 g chloralose Heart volume Ten milligrams of the alkaloid into the femoral vein show an increase in the volume of the heart Time=6 seconds

(*d*) Male cat 3 420 g chloralose Pulmonary pressure and carotid blood pressure Injection of 10 mg of the *Moringa* alkaloid into the femoral vein produce an increase in the pressure of both The rise in pulmonary pressure is more sustained Time=6 seconds

(*e*) Female cat 2 645 g chloralose Kidney volume and carotid blood pressure, showing effects of injection of 10 mg of the *Moringa* alkaloid Time=6 seconds

(*f*) Male cat 1 600 g chloralose Intestinal volume and carotid blood pressure showing effects of injection of 7 5 mg of the *Moringa* alkaloid Time=6 seconds

(*g*) Perfusion of isolated heart of kitten 1 100 000 of the *Moringa* alkaloid produce an increase of the frequency and force of the beats Upstroke diastole and downstroke systole Time=6 seconds

(*h*) Perfusion of virgin uterus of rabbit with total alkaloid of *Moringa* 1 40,000, shows contraction and increase of tone Time=6 seconds

(*i*) Perfusion of isolated uterus of guinea pig (early pregnancy) with total alkaloid of *Moringa* 1 10,000 Shows contraction Time=6 seconds

amplitude of the beats of the auricles, the ventricles showing practically little or no response On repeated administration of large doses, after a total of 80 to 100 mg is given, the blood-pressure begins to show a fall before the usual rise If still larger quantities are given, it is observed that as the doses are increased, the initial fall of blood-pressure which occurs is more marked while the ultimate rise produced is less When a total of 199 5 mg has been given, the blood-pressure not only shows no rise but actually falls (Graph 2, *a*) At this stage a dose of 1/150 c c of adrenalin produces a fall in blood-pressure and slight dilatation of the ventricle (Graph 2, *b*) while 1/20 c c of adrenalin produces a rise which also is much smaller than that produced normally When the animal is allowed to recover from the effects of the alkaloid, doses of 1/150, 1/200, 1/400 and 1/500 mg of adrenalin produce a rise of blood-pressure and the usual effect of the drug on the contractions of the auricles and the ventricles Further it is observed that when large doses of the alkaloid are repeatedly given, the auricles and ventricles show the usual effects of working against increased peripheral resistance, during the period of the high blood-pressure As the doses are gradually increased and the blood-pressure begins to show a larger initial fall and a smaller subsequent rise, the effects on the auricles and on the ventricles are correspondingly less Lastly, when sufficient doses have been given so that only a fall of blood-pressure is produced, the contractions of the auricles and ventricles show little or no change These facts show that large doses of the alkaloid have no depressing effect either on the myocardium or on the accelerator nerves in the heart The fall of blood-pressure is, therefore, due to changes in the calibre of the blood vessels Furthermore, it has been observed that administration of small doses of adrenalin after such repeated doses of the alkaloid produces a fall of blood-pressure and a slight dilatation of the ventricles Under the conditions stated, such an action of adrenalin is only possible when either the musculature of the vessel wall is depressed or the vaso-constrictor sympathetic fibres are inhibited, leaving the vaso-dilators unaffected It would appear, therefore, that repeated large doses of this alkaloid produce no effect on the myocardium, but either depress the musculature of the blood vessels or the vaso-constrictor sympathetic fibres As however the drug does not depress the cardiac muscle, it is not likely that it would depress the musculature of the vessel It appears probable that the drug in large doses depresses the vaso-constrictor fibres

Volume of the organs —The volumes of different organs react in different ways to this drug, and these changes are difficult to interpret Generally the changes in the volume are attributed to alteration in the calibre of the blood vessels in the organs, or to contraction of involuntary muscle fibres in the capsule, or both It has been shown that this alkaloid in doses of 10 mg produces a reduction in the volume of the spleen which corresponds to the rise of blood-pressure (Graph 3, *a*, *b*), and the volume of the intestine shows an initial reduction followed by an increase

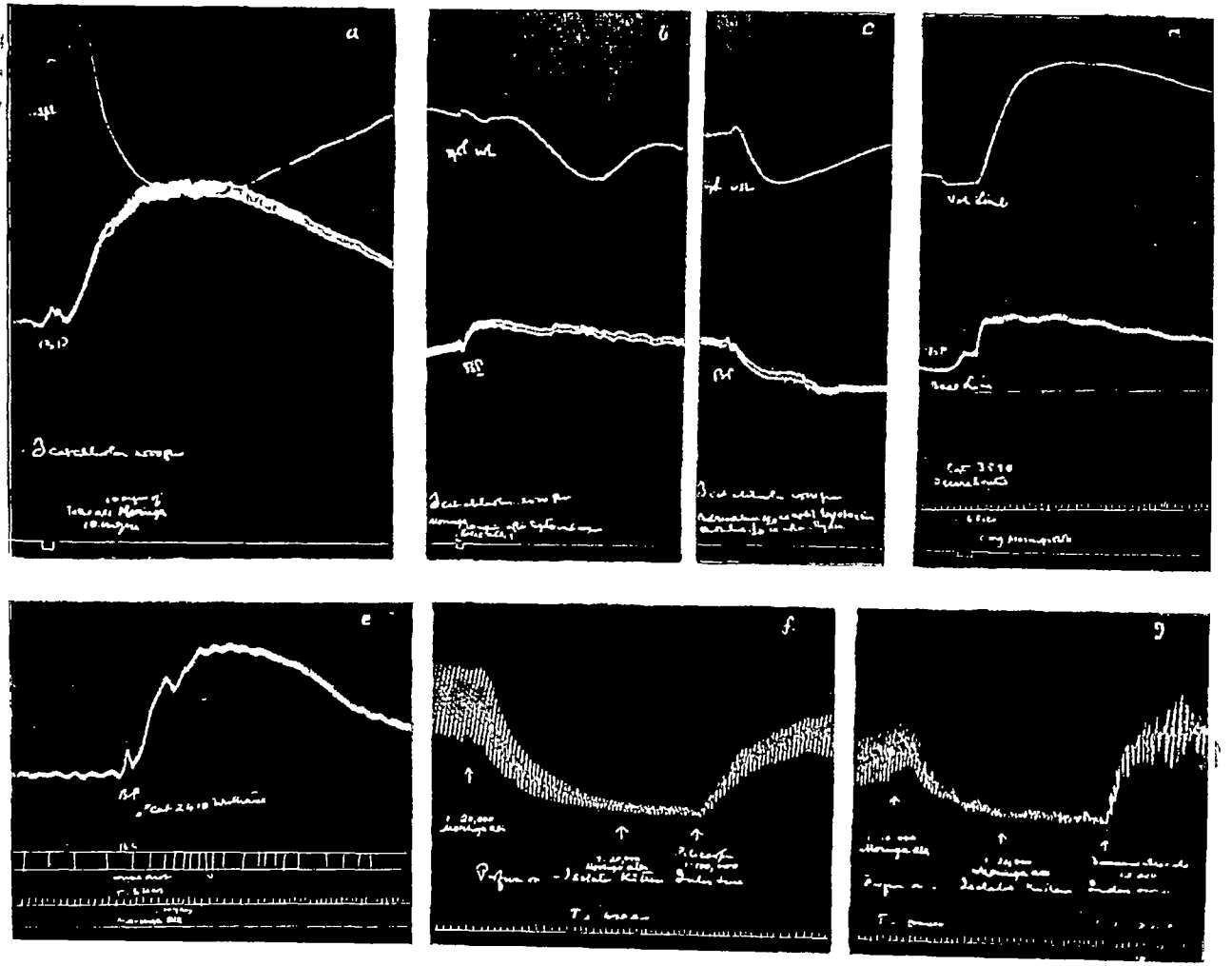
(Graph 1, *f*) This increase in volume persists for some time after the carotid blood pressure comes down to normal The kidney (Graph 1, *e*) and the limb volumes (Graph 3 *d*) also show a rise, the kidney volume sometimes shows a tendency to initial constriction The reduction in the volume of the spleen is due to contraction of the involuntary muscle fibres in the organ, the dilatation seen in the other organs is probably produced by their passive engorgement with blood on account of the constriction of the vessels elsewhere, stimulation of the heart and rise of the blood-pressure Though the volumes of some of the organs show a passive dilatation the volumes of the intestine and the kidneys at any rate show an initial reduction (corresponding to the early part of the rise of the blood-pressure) followed by an increase The marked and persistent increase in the volume of the intestine, seen after the return of the blood-pressure to normal, may be partly due to inhibition of the tone and consequent relaxation of the intestine produced by this alkaloid It was further observed that after administration of repeated doses of ergotoxin, which paralyse the vaso-motor sympathetic fibres, the volume of the spleen still shows a fair reduction though it is much smaller than that observed when ergotoxin is not administered (Graph 3, *a*, *b*) This shows that the action of this alkaloid is both on the musculature of the organ and the vaso-motor sympathetics The initial reduction of the intestinal volume seen with this alkaloid is practically absent after administration of ergotoxin

Digestive system —Intravenous injections of the alkaloid in doses of 5 to 10 mg produce a general inhibition of the tone and movements of the intestine *in situ* Sometimes a complete inhibition of the intestinal movements lasting from 3 to 10 minutes is produced (Graph 2, *d*) Perfusion of the isolated pieces of the gut produces a distinct reduction in the amplitude of peristaltic movements and inhibition of the tone Further, it is observed that after perfusion of the intestine with repeated large doses of the alkaloid, pilocarpine and barium chloride produce their usual effects (Graph 3, *f*, *g*) This shows that this alkaloid does not paralyse the vagus nor does it depress the musculature of the intestine The effect must, therefore, be on the sympathetic nerves

Liver —The alkaloid when injected through one of the mesenteric veins produces only slight effects, and when the injection is given slowly no effect whatsoever is produced It would appear from this that the liver either retains the alkaloid or prevents it from passing into general circulation in active form

Respiratory system —The effect of the drug on the respiration was studied by observing the intra-tracheal pressure in cats under urethane anæsthesia Intravenous injections of the alkaloid produce a marked fall in the intratracheal pressure (Graph 2, *c*), pilocarpine produces its usual effects afterwards The effects produced by the alkaloid are present in a normal degree in atropinized animals, and pilocarpine produces its normal effect after its administration It is probable, therefore, that the action is not on the vagi In some animals a slight initial constriction of

GRAPH 3



(*a*) Male cat, 2,500 g, chloralose Upper tracing shows the spleen volume and the lower the carotid blood pressure after injection of 10 mg of the *Moringa* alkaloid Spleen volume shows reduction

(*b*) Same as (*a*) but the alkaloid was given after administration of sufficient ergotoxin to paralyse the sympathetics Shows a lesser effect than (*a*)

(*c*) Same as (*b*), an injection of 1/20 c c adrenalin was given to compare its effect with that of the *Moringa* alkaloid after ergotoxin Interval between (*b*) and (*c*)=1 minute

(*d*) Male cat, 3,570 g, chloralose Limb volume and carotid blood pressure Shows effect of injection of 10 mg of the *Moringa* alkaloid Time=6 seconds

(*e*) Male cat, 2,410 g, urethane Blood pressure and urine drops indicated by vertical strokes Injection of 5 mg of the alkaloid shows an increase in the flow of urine corresponding to the rise of blood pressure Time=6 seconds

(*f*) and (*g*) Isolated intestine of kitten, 1 20,000 solution of the *Moringa* alkaloid produces a relaxation of the tone and diminution of the movements of the intestine Also shows that pilocarpine and barium chloride had usual effects after *Moringa* alkaloid Time=6 seconds

the bronchioles is observed after administration of the alkaloid, and this constriction is more marked when it is given after an injection of adrenalin

Genito-urinary system —The effect of the alkaloid on the secretion of urine was studied on cats under chloralose or urethane anæsthesia A suitable tube was introduced into the bladder by supra-pubic operation and the drops of urine were recorded The secretion of urine was slightly increased, after intravenous injections of the alkaloid, and the effect lasted as long as the blood-pressure kept at a raised level (Graph 3, *e*) The kidney volume always showed an increase corresponding to the rise of blood-pressure (Graph 1, *e*) It can, therefore, be suggested that the increase in the secretion of urine is the result of the high blood-pressure and consequent increase of blood flowing through the kidneys This is borne out by the fact that if both the kidneys are encased in a plaster of paris jacket, so that they cannot increase in volume, there is no increase in the secretion of urine after injections of this drug

Uterus —That the sympathetic contains both the augmentor as well as the inhibitory fibres of the uterus is an accepted view, though some doubt has been thrown on it on account of the different responses given by nicotine, hydrastinine, etc , in intact and isolated uteri of the cat The uteri of different animals in different conditions respond differently to the sympathomimetic substances Advantage was taken of this behaviour of the uterus to determine the exact nature of action of this new alkaloid on this organ Experiments have shown that adrenalin contracts the uteri of rabbits and relaxes those of guinea-pigs Isolated rabbit's uterus, both virgin and parous as well as the pregnant uterus of the guinea-pig, all respond by contraction to 1-40,000 dilution of this alkaloid (Graph 1, *h*, *i*) This at once throws doubt on the idea that the alkaloid acts on the sympathetic exclusively On further investigation it is found that the action of the alkaloid on the rabbit's uterus is not altered after previous treatment of the uterus with ergotoxin (as is the case with adrenalin) This shows that the action on the uterus is not due to the stimulation of the sympathetics but to its direct action on the muscle It has been further observed that atropine (1-75,000) has no action on the uterus of the guinea-pig contracted with 1-40,000 of this alkaloid, whereas solution of adrenalin (1-1,000,000) relaxes it

Discussion

The important effects of this alkaloid are a rise of blood-pressure, inhibition of the tone and movements of the plain muscles of the intestine and relaxation of the bronchioles These effects are produced by many sympathomimetic bases like adrenalin, ephedrine, etc The effects of sympathetic stimulation are also evident in other organs The alkaloids *M pterygosperma* stimulates the smooth muscles, and this is clearly seen in the case of the uterus, while the cardiac muscle is only slightly stimulated This alkaloid, though it closely resembles adrenalin

in its action, differs from it in that it produces some rise of systemic blood pressure after ergotoxin, whereas adrenalin produces a fall Ephedrine in large doses depresses the myocardium, whereas this alkaloid has no such depressant action The comparative effect of the three bases is seen in the table *M pterygosperma* alkaloid is weaker than ephedrine or adrenalin

It is interesting to note that the sympathetic action of this alkaloid predominates in case of the intestines and the bronchi, whereas the muscular action predominates in the uterus In the circulatory system the action is both on the sympathetic and on the muscle

The discovery of these alkaloids is yet another instance of a strong sympathomimetic base being present in medicinal plants commonly used in indigenous medicine We have already published our results (Chopra and De, 1930) of the presence of ephedrine in *S cordifolia* The comparative effects of *Moringa* alkaloid, ephedrine, and adrenalin on the different systems are given in the following table

TABLE

	Moringa alkaloid	Ephedrine	Adrenalin
Blood pressure	Prolonged and persistent rise, rarely comes down below normal	Sharp and persistent rise	Sharp rise quickly followed by a fall sometimes below normal
Pulmonary pressure	Slight increase	Slight increase	Marked increase
Coronary outflow	Increased slightly	Increased	Increased
Myocardium	No depression after repeated doses	Depression after repeated doses	Little or no action
Blood vessels	Constriction	Constriction	Constriction
After ergotoxin	Little or no rise of blood pressure	Little or no rise of blood pressure	Fall of blood pressure
Intestinal movement	Inhibition	Persistent inhibition	Inhibition
Respiration	Relaxation of bronchioles	Relaxation of bronchioles	Relaxation of bronchioles
Uterus	Contraction of uterus of rabbit and guinea-pig	Contraction of uterus of rabbit	Contraction of uterus of rabbit and relaxation of that of guinea pig
Seat of action	Chiefly on sympathetic nerve endings and slightly on musculature of the heart directly and other plain muscles	Chiefly on sympathetic endings	Chiefly on sympathetic nerve endings

Summary and conclusion

1 The alkaloid of *M pterygosperma* acts on the sympathetic nerve-endings as well as on the cardiac and smooth muscles all over the body It produces a rise of blood-pressure, stimulation of heart and contraction of blood vessels It also relaxes the bronchioles, inhibits the tone and movements of the intestines and contracts the uterus in guinea-pigs and rabbits

2 The alkaloid produces a slight diuresis due to rise of blood-pressure

3 The alkaloid is detoxicated by the liver

4 In large doses only the sympathetic motor fibres of the vessels are depressed by the alkaloid

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position, when the major portion of the supply is obtained from the Ghazipur opium factory, a small quantity from Kulu and Kothkhai and a still smaller quantity from Simla Hill States

Another source of supply, though illegal, cannot be overlooked as its extent is not inconsiderable, and that is smuggling In spite of the precautions and strict vigilance of the excise department, quantities of opium appear to pass into the province from Rajputana States, Simla Hill States and from the tribal territory in the North-West Frontier and Afghanistan The smuggling from the Hill States is possible owing to the very low price of opium in those areas, the high limit of private possession allowed and the comparative freedom from control licenses The geographical situation of the Punjab makes it easily accessible from all sides, and as poppy is cultivated in various places in its neighbourhood smuggling of opium can be easily carried out Simla Hill States produced opium, and this could without difficulty find its way through a hundred bye-paths into Patiala State and from there to other States adjoining British districts Opium from Jammu and Kashmir State used to get into Sialkot Gurdaspur and other sub-montane districts, but this has now completely stopped as cultivation of poppy and production of opium are prohibited in the State The produce of the independent tribal territories on the border of the Hazara district in the North-West Frontier Province has not far to travel before it can reach Hazara and the Attock districts

Control of opium in the Punjab — As regards the sale of opium to the public the policy of the Punjab Government is in accord with the policy of the Government of India, that is non-interference with the moderate use of raw opium, whether the object of the consumer be some real or supposed physical benefit, or the desire for a stimulant or a narcotic The only noteworthy feature is stringent restriction imposed on opium smoking As early as 1890 the Government of the Punjab placed restriction on opium smoking, and in 1923 passed the 'Punjab Opium Smoking Act of 1923' The Government wished to restrict opium eating as well as opium smoking, but in regard to the latter the regulations imposed were so stringent that the restriction amounted practically to total prohibition The Opium Smoking Act of 1923 prohibits opium smoking in assemblies, in all municipalities and cantonments The members of assemblies, and the owners and managers of places used for opium smoking were made liable to penalties under the Act Smoking mixtures containing opium cannot be sold by licensed vendors of opium or by any other person The smoker must prepare his own smoking mixture, and must not have in his possession more than half a tola of the mixture at one time

As regards opium eating the Government is undoubtedly anxious to prevent its misuse and excess[illegible] umption This is being effectively done by increasing t[illegible] opi[illegible] dually decreasing the supply

The sources of supply of opium in the Punjab—It has already been indicated that the Punjab differs from other provinces of India in that it derives a part of its supply of opium from sources other than the Government factory at Ghazipur Most of the opium consumed at the present time, however, is supplied by the Government factory through district excise authorities 'Home grown' opium (i e, opium grown in Kotkhai and Kulu and Hill States opium) was formerly allowed to be sold in the Lahore district through licensed vendors who transported and imported it under Government permits and this practice is still allowed in Jullundur division A perusal of Table II will show the amounts of different kinds of opium used in the Punjab between 1900-01 and 1929-30 It will be observed that opium obtained from all sources shows a marked decrease

TABLE II

Showing the consumption of different kinds of opium in the Punjab from 1900-01 *to* 1929-30

Year	Ghazipur excise opium	Malwa opium	Afghan opium	Hill States opium	Home grown opium	Total of other than excise opium	GRAND TOTAL
1900-01	9,912	16,859		13,944	16,586	47,389	57,301
1901-02	7,858	16,764		9,538	18,638	44,940	52,798
1902-03	8,461	16,599		7,153	19,781	43,833	52,294
1903-04	8,809	15,357		5 604	24,075	45,036	53,845
1904-05	7,014	10,428		12,534	27,757	50,719	57,733
1905-06	8,632	19,603		10,911	19,674	50,188	58,820
1906-07	10,207	24,737		5,834	19,129	49,700	59,907
1907-08	9,574	29,813		8,497	12,926	51,236	60,810
1908-09	18,154	11,126		14,063	18,072	43,261	61,415
1909-10	31,902			10,544	17,059	27,603	59,505
1910-11	44,049			10,186	9,137	19,323	63,372
1911-12	52,501			6,548	4,432	10,980	63,481
1912-13	57,634		847	4,652	3,451	8,950	66,584
1913-14	54,731		156	6,729	3,733	10,618	65,349
1914-15	55,144		47	2,005	2,625	4,677	59,821
1915-16	54,685		5	2,012	2,371	4,418	59,103
1916-17	51,007		203	7,954	1,990	10,147	61,154
1917-18	50,445		210	5,633	1,472	7,315	57,760
1918-19	48 008		6	3,898	1,460	5,364	53,372
1919-20	44,683			4,427	2,248	6,675	51,358
1920-21	37 225			11 621		11,621	48,846
1921-22	23,893			13,902		13,902	37,795
1922-23	25,285			9,514		9,514	34,799
1923-24	28 234			5,143		5,143	33,377
1924-25	29,872			3,810		3,810	33,682
1925-26	34 929			2,727		2,727	37,656
1926-27	36,278			2,845		2,845	39,123
1927-28	37,638			2,918		2 918	40,556
1928-29	37,048			2 279		2 279	39,327
1929-30	36 922			2,021		2,021	38,943

Consumption of opium in the Punjab —A reference to Table II will show that in 1929-30, 38,913 seers of opium were consumed in the province, out of which 36,922 seers were excise opium while 2,021 seers were 'home grown' opium These figures do not include the quantity sold by pharmaceutical chemists in the form of medicinal preparations, or dispensed in prescriptions or preparations supplied to Government medical institutions by the Government Medical Store Depôts

The consumption of raw opium in the province can be discussed under three main headings —

(*a*) Medical use—(1) for human beings, (2) for animals
(*b*) A household remedy
(*c*) A drug of addiction

(*a*) *Medical uses of opium* —There is no doubt that opium is one of the most useful drugs in the Pharmacopœia, and it is extensively used in western medicine Although no statistics are available to show how much opium or its derivatives are used by medical practitioners in this country, it is certain that the quantity consumed in this way is not large From our work in the field it is clear that little or no opium is sold by ordinary licensed vendors to the registered medical practitioners There is reason to believe that the Indian medical practitioners use less opium and its derivatives than their western confrères This contention is supported by the fact that in the Punjab we have not come across a single individual who could say that his habit had followed taking of opium on medical advice

Chopra (1928) gave a detailed account of the use of opium in the indigenous systems of medicine in India He showed that opium is not used to any great extent in Hindu medicine Its administration is mainly confined to two diseases, namely, diarrhœa and dysentery, and it is only given in certain stages of these conditions The Hindu physicians appear not to have made much use of the sedative and pain-relieving properties of opium and, even at the present time, they only use it occasionally to relieve pain and spasms Our investigations in the Punjab show that very little opium is being used by the practitioners of indigenous medicine, but there is little doubt that itinerant quacks give it in all sorts of diseases and conditions to those who go and consult them

It has been said that large quantities of opium were used for purposes of administration to domestic animals Our investigations in the province show that, though the custom might have been prevalent in the days when poppy was commonly cultivated everywhere, it is not met with now Because of the high price of opium and the large doses that are necessary for animals, the expense would be prohibitive at the present time Many years ago it was not uncommon for the

hackney carriage drivers to give the drug to the horses in order to overcome fatigue and to make the animals endure continued physical strain On account of the high price of opium this is rarely done now The drugs employed for this purpose in these days are compounds of arsenic, various preparations of *Cannabis indica* (e g , bhang, charas, etc), strychnine, flowering tops of *Calotropis gigantia* and opium only to a very small extent When opium is used for this purpose it is administered once a day, generally in the evening, in doses of one-quarter to one tola After the dose the animals look active, can undertake long journeys, and take fodder well Animals who are given daily doses become habituated to the drug , we have met a few instances of this condition When opium is withheld from these animals they show a train of symptoms resembling those occurring in drug addicts They lose their appetite, their eyes water, they look off colour and show signs of tiredness when the dose is not given They become lazy and obstinate and will not move Such animals have a peculiar appearance, are thin and emaciated, and have really become drug addicts

Opium as a household remedy —A reference has also been made to the use of opium as a household remedy in India generally It has been shown that despite the observations made by Dr Roberts in the Report of the Royal Commission of 1893, the use of opium as a household remedy in India is very limited at the present time So far as the Punjab is concerned, opium has never been used as a prophylactic against malaria, or to keep off damp and cold It is used to a certain extent to control intestinal fluxes, and for coughs and colds in the winter season, but that also in very small quantities One meets people who have passed the age of 45 or 50 taking half a grain of opium daily during the winter months to keep down cough and bronchial secretion, but even this is getting less common than in days gone by

It has been alleged that the use of opium as a household remedy in the Punjab is due to insufficient medical relief being available for the masses, especially in the rural areas Our investigations have shown that there is no correlation between the number of dispensaries and opium addicts in an area The consumption of opium is highest in the central districts, e g , Ferozepur, Lahore, Ludhiana and Jullundur, which are well provided with medical facilities The average area served by a dispensary in the province as a whole is 287 square miles, with an average population of 62,643 The average in Mianwali district is 597 square miles and in Jullundur 96 square miles (*Annual Statements of the Dispensaries and Charitable Dispensaries of the Punjab*, 1924) One would expect that in Mianwali district, where facilities for medical aid are comparatively less, the consumption of opium would be more, but it is about 6 seers (12 pounds) per 10,000 of population (i e , in accordance with the standard laid down by the League of Nations as being necessary for the medical and scientific needs of the people), whereas in Jullundur it is 24 seers per 10,000, i e , 4 times as much

From these and other facts it is obvious that most of the raw opium in the Punjab is consumed by the addicts Recognition of this position is of importance, as further measures adopted to restrict or abolish sale of raw opium depends on this

The present extent of opium addiction in the Punjab —The average consumption of opium in the Punjab in 1929-30 was 0 18 seers per 100 of the population The League of Nations has roughly estimated the consumption of opium of a country for medical and scientific needs as 0 06 seers per 100 of the population If we study the state of affairs in the Punjab as a whole, with reference to the standard laid down by the League of Nations, the consumption of opium is not excessive A closer study, however, shows that this comparison does not give a correct idea of the extent of the use of this drug among different sections of people and in different localities We have carefully studied the figures of consumption of opium in various districts and the results are shown in Table III Seven districts of the Punjab (with a population of 22 9 per cent of the total) show a consumption either below or at the level of the standard laid down by the League Seven districts with a population of 21 7 per cent are not much above the standa laid down by the League Seven districts (with a population of 19 3 per ce the total) consume less than the average for the province i e 0 18 seers p In four districts (with a population of 18 01 per cent of the total) the con is very high These are the central districts, Ferozepur—with a c of 0 54 seers per 100 of population, Lahore—with 0 49 seers per 100 0 48 seers per 100, and Amritsar—with 0 42 seers per 100 of p remaining four districts of the province (with a population of 1 a higher consumption than the average for the province with 0 24 seers per 100, Ambala with 0 23 seers per 100, L per 100 and Rawalpindi with 0 18 seers per 100 of popula

It will be seen, therefore, that the problem of opium when the province is considered as a whole onti habit is not widespread in this part of Ind giving rise to the high incidence of opi consumption in such parts is dep area rather than on such factor etc In the rural areas in the between the consumption of opi of a locality The districts wit incidence of addiction, as com population, which show a cons however, hold good for the ru the Sikh element is not large of opium addiction in the ru

TABLE III

Showing the consumption of opium in various districts of the Punjab in 1901-02, 1913-14 *and* 1929-30

Name and group of districts		Population according to 1921 census	Percentage of the total population of the province	Total consumption of opium in seers during			Consumption of opium in seers per 100 of population during			Percentage variation between 1901-02 and 1929-30
				1901-02	1913-14	1929-30	1901-02	1913-14	1929-30	
I Group —										
Ferozepur	Area with high consumption	1,098,248	18 01	8,003	12,471	4,717+1,273	0 83	1 29	0 54	−34 8
Lahore		1,131,336		5,415	7,820	5,505+ 89	0 46	0 75	0 49	+ 6 5
Ludhiana		567,622		3,669	4,663	2,641+ 95	0 54	0 81	0 48	−11 5
Amritsar		929,374		6,226	7,057	3,826+ 110	0 65	0 8	0 42	−30 0
		3,726,580								
II Group —										
Lyallpur	Consumption just above average for the province	979,463	18 1		2,655	2,216		0 29	0 22	
Jullundur		822,544		4,046	3,143	1,819+169	0 44	0 39	0 24	−45 4
Ambala		631 477		3,090	2,960	1,627]	0 37	0 41	0 23	−37 7
Rawalpindi		569,224		1,618	2,082	1,067	0 17	0 38	0 18	+ 5 8
		3,052,708								
III Group —										
Simla	Consumption just below average for the province	45,327	19 3	321	353	80	0 79	0 89	0 17	−78 5
Hoshiarpur		927,419		2,018	2,125	905+229	0 20	0 23	0 12	−40
Gurdaspur		852,192		2,480	1,517	1,084+ 6	0 26	0 18	0 14	−46 1
Gujranwala		623,581		1,796	1,943	977	0 23	0 21	0 15	−34 7
Sheikhupura		523,135				1,017+ 3			0 17	
Montgomery		713,786		839	832	1,205	0 18	0 16	0 17	− 5 5
Multan		890,264		1,401	1,910	1,416	0 19	0 23	0 15	−20
		4,575,704								

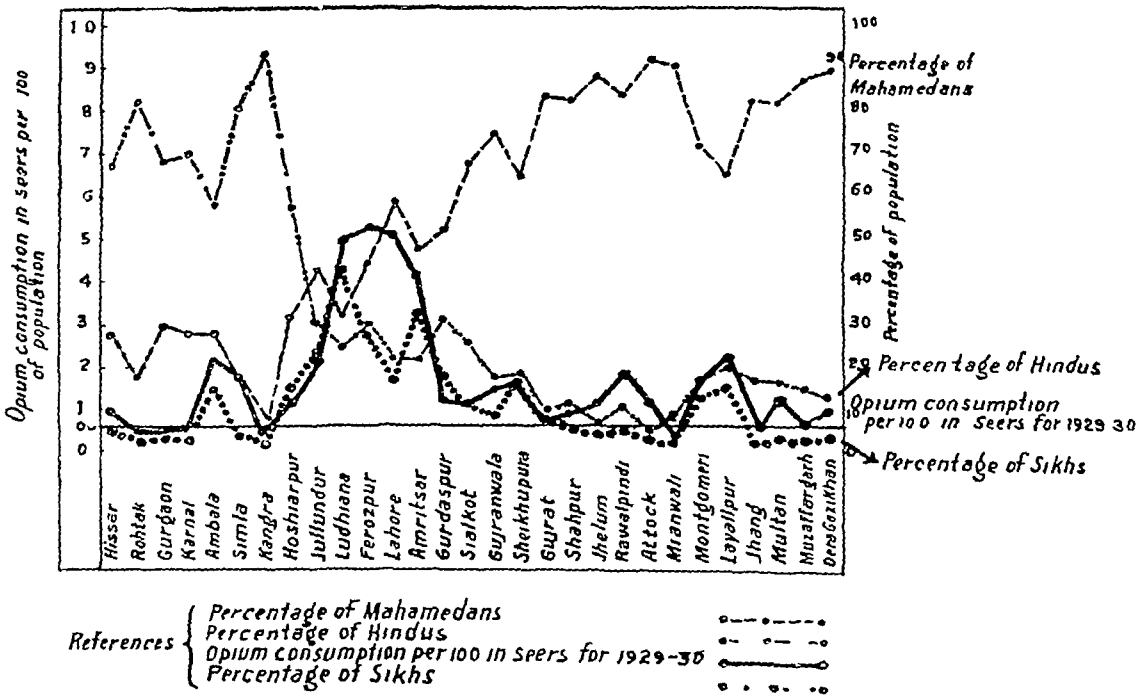
TABLE III—*concld*

Name and group of districts		Population according to 1921 census	Percentage of the total population of the province	Total consumption of opium in seers during			Consumption of opium in seers per 100 of population during			Percentage variation between 1901-02 and 1929-30
				1901-02	1913-14	1929-30	1901-02	1913-14	1929-30	
IV Group —										
Hissar	Consumption just above League of Nations' Standard	816,810	21·7	952	3,922	777	0·12	0·47	0·03	−10·0
Sialkot		937,823		1,388	1,119	959	0·13	0·13	0·10	−23·1
Shahpur		719,918		354	640	684	0·06	0·09	0·09	+50·0
Jhelum		477,068		512	785	566	0·09	0·15	0·11	+22·2
Jhang		570,559		1,558	504	413	0·13	0·09	0·07	−46·1
Dera Gazi Khan		495,810		309	895	440	0·06	0·18	0·09	+50
Attock		512,249			689	547		0·1	0·13	
		4,530,237								
V Group —										
Rohtak	Consumption at or below League of Nations' Standard	772,272	22·9	440	711	321	0·06	0·9	0·04	−33
Gurgaon		682,003		991	917	322	0·13	0·12	0·04	−67
Karnal		828,726		1,440	1,272	500	0·16	0·15	0·06	−62
Kangra		766,065		430	585	1904-63	0·05	0·07	0·03	−40
Gujrat		824,046		449	516	520	0·05	0·06	0·06	+20
Mianwali		358,205		226	200	237	0·05	0·05	0·06	+20
Muzzafargarh		568 478		534	750	335	0·13	0·13	0·05	−61
		4,790,795								
	TOTAL	20,685,024	100	52,798	65,366	36,022+2,021	0·26	0·33	0·18	−30·7

the towns—owing to overcrowding and other factors which have been discussed later—the lower strata of society are much more prone to take opium than in villages where these conditions do not exist The correlation which exists between consumption of opium and percentage of Sikhs in the population of a locality is shown in Graph 1 The variation in the percentage of the population of different communities and the consumption of opium in seers per 100 of the population in the various districts is depicted in the form of curves The graph is self-explanatory

GRAPH 1

Showing percentage of population of different communities in different districts of the Punjab with incidence of opium consumption per 100 *of population during the year* 1929-30



The opium curves shows four peaks indicating increased consumption The first peak is over the district of Ambala where the curve of the Sikh population also shows a corresponding rise The rise of opium consumption runs almost parallel with the rise in the percentage of the Sikhs in the population of the districts of Hoshiarpur, Jullundur, Ludhiana and Amritsar This correspondence of the two curves, however, is not so exact in case of Ferozepur and Lahore districts The cause of this discrepancy here is in all probability that in these districts the Hindu,

Sikh and Mohammedan population curves tend at practically the same level, unlike most of the other districts where there is a great preponderance of Mohammedans In case of Lahore district the presence of the large towns of Lahore and Kasur with a large mixed population is also responsible Just as opium consumption figures for the whole of the Punjab do not give a true picture of the consumption of opium among various sections of the population and localities in the same way the consumption figures of the whole of the districts of Lahore and Ferozepur might present a somewhat misleading picture

We have gone into the consumption figures of the tahsils of three districts and find that the correlation between Sikh population and opium consumption above indicated is quite correct This will at once be obvious from a perusal of Table IV

TABLE IV

Showing the percentage of population and the consumption of opium in seers per 100 of population in the tahsils of Ferozepur, Lahore and Ludhiana

Name of tahsil	Consumption of opium in seer per 100 of population	Percentage of population		
		Sikhs	Hindus	Mohammedans
FEROZEPUR DISTRICT —				
Moga	1 0	56 13	19 83	24 04
Muktsar	0 38	31 63	26 00	42 37
Ferozepur	0 51	27 15	21 27	51 28
Zira	0 10	21 11	16 35	63 54
Fazilka	0 20	13 15	13 93	42 62
LAHORE DISTRICT —				
Kasur	0 60	23 06	17 98	58 06
Chunian	0 28	17 0	21 2	61 8
Lahore	0 57	12 7	28 3	59 0
LUDHIANA DISTRICT —				
Jagroan	0 60	48 2	17 4	34 4
Ludhiana	0 42	40 3	24 6	35 1
Samrala	0 21	36 7	31 7	31 6

N B —Tahsil is a portion of a district for administrative purposes One district may contain several tahsils

The percentage of Sikhs is highest (56 13 per cent) in Moga tahsil of Ferozepur district and it shows a consumption of one seer per 100 of population, whereas the Fazilka tahsil of the same district has a percentage of 13 45 of Sikhs and shows the consumption to be only 0 20 seers per 100 of the population Again the Chunian tahsil with 17 per cent of Sikh population shows a consumption of 0 28 seers per 100, while Kasur tahsil of the same district with 23 96 per cent shows a consumption of 0 6 seers per 100 Jagraon tahsil, with 18 2 per cent of Sikh population, shows a consumption of 0 6 seers per 100, and Samrala tahsil, with 36 7 per cent of Sikh population, shows only 0 21 seers per 100 The other two small rises in consumption of opium are over Rawalpindi district and Lyallpur district and these correspond more or less with the rise of the curve of the Sikh population

It will be seen, therefore, that the Sikh community as a whole is much more addicted to the use of opium than other communities in the Punjab It is very difficult to assign any definite reasons for this high incidence of consumption among these people Attempts have been made to show that the opium habit was common in those areas where the drug was grown, and that as it was chiefly the agriculturists who grew opium they became addicted to the drug The Sikhs being an agriculturist class were, therefore, larger consumers of opium This explanation may appear to be plausible, but a closer examination shows that it is not borne out by facts Thus, in Ferozepur and Ludhiana districts there is a large Sikh population who have never cultivated poppy on a large scale, and yet they show the highest consumption in the province The best opium-producing district was Shahpur with a high percentage of Mohammedans, and this shows a very low consumption The probable explanation of this high consumption is that the Sikhs, being a martial race, have undergone terrible hardships and physical strain during the last century and a half They learnt the use of the drug during this period of adversity and have kept it up It is worthy of note in this connection that the Rajputs, who are also a martial race, are greatly addicted to this drug, according to some authorities they consume much larger quantities than the Sikhs It has also been observed that the use of intoxicants and similar drugs always shows increase during and after the period of wars It must be stated, however, that many of the other martial races in India such as the Punjabi Mohammedan, Pathans, Gurkhas, Dogras, etc, do not use opium or other intoxicants

Is opium addiction on the increase ? The only practical method at our disposal to determine the increase or decrease of opium addiction in the province is from the study of the figures of the sale of opium in the excise reports This, however, is only a rough method The accurate method would be to count the addicts during a census, which for obvious reasons is not a practical proposition A study of the excise statistics of the Punjab from 1901–1930 shows that the consumption of opium in the province has fallen by 31 per cent A perusal of Table II shows that up to the year 1912-13 the consumption of opium was on the increase, and

that in this year it reached its highest, namely 66,581 seers This means an increase of 2 1 8 per cent over the figures of 1901-02 From that year the consumption has declined steadily though 1916 17 showed increased consumption over the previous year In the year 1921-22 there was a sudden drop of 21 per cent from the figures of the preceding years It will serve no useful purpose to enter into details as to the causes which gave rise to large fluctuations in the figures of opium consumption in the various districts of the Punjab, for the excise figures are vitiated by two factors —

Firstly, the statistics before 1909, when 'home grown opium was largely used, are vitiated by a lack of uniformity in the district returns Ambala and Karnal districts produced soft opium, which lost half of its weight during its preparation for consumption In certain districts the actual weight of this opium is returned as consumed, and in others the weight of the prepared opium is returned as consumed, thus introducing an error In the same way Shahpur and Malwa opium lost 38 per cent of its weight by drying In former days, 'pukka' opium was prepared before use This was done by boiling opium with water, any scum rising on the surface was removed and the fluid opium decanted and passed through fine cloth and then boiled again to a thick consistency This preparation introduced a slight error the extent of which is difficult to ascertain

Secondly, smuggling of opium into the province has constantly been going on In the *Excise Administration Reports of* 1923-24 the following passage occurs — 'Hitherto attention has been fixed on this remarkable reduction in the consumption of excisable articles, but along with this the increase at a rapid rate of the illicit and smuggled articles has not been sufficiently noticed Consequently there has been a tendency to a complacent satisfaction at the supposed improvement in the habits of the people The facts disclosed in the report as to the existence of illicit distillation and smuggling indicate that there is less reason for satisfaction' This factor is by no means small, though the extent to which it vitiates the figures is difficult to ascertain with any degree of accuracy

Taken as a whole, the figures, as they stand, show a drop of 31 per cent in the total consumption of opium in the province from 1900-01 to 1929-30 In Graph 2 we have recorded the consumption of opium per 100 of population in 1901-02, 1913-14 and 1929-30 The progressive decline in opium consumption after 1913-14 is quite obvious This fall is undoubtedly due to the measures introduced by the authorities from time to time to reduce excessive consumption in accordance with the general policy agreed to by the League of Nations These measures are —

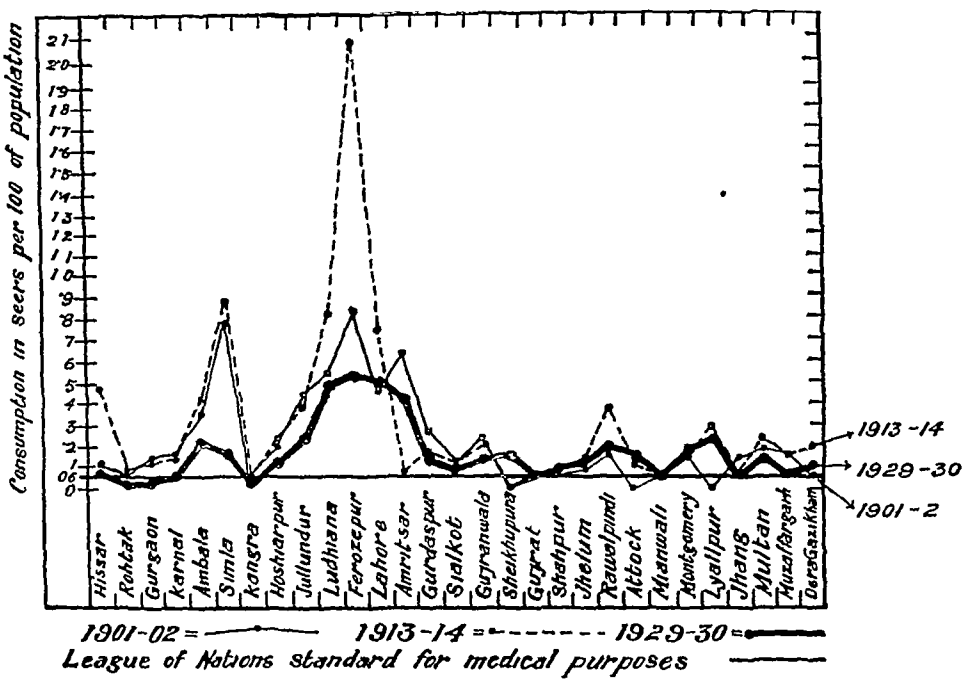
(1) The limitation of poppy cultivation The cultivation of the poppy has been gradually cut down in the Punjab The growing of the poppy for production of opium is nowadays entirely limited to a small area in the Simla Hills The production and preparation of opium has been centralized, and the Central Government undertake to manufacture and distribute it to various provincial governments

according to their requirements This has stopped promiscuous cultivation of the poppy in rural areas, and has in this way removed from the doors of the rural population the temptation of eating opium

(2) The increase in the retail price of opium The actual sale price has increased from an average of Rs 25-14-4 per seer in 1901-02 to an average of Rs 100 per seer in 1929-30

GRAPH 2

Consumption of opium in the Punjab in seers per 100 *of population in the different districts during the years* 1901-02, 1913-14 *and* 1929-30



(3) The introduction of the system of 'rationing' in the issue of opium to vendors This has greatly helped in keeping down consumption According to this system the vendor cannot get more than a specified quantity of opium, which has been worked out from a careful survey of the local requirements This prevents the vendors from encouraging the use of opium in the locality in which the shop is situated, or selling it in large quantities to the consumers outside the area allotted to them

(1) The decrease in the number of retail shops This makes the drug more difficult to procure The number of licensed shops selling retail opium was 1,156 in 1901-02, and in 1929-30 this was reduced to 671

It has been pointed out that these measures have resulted in reducing the total consumption of opium by 31 per cent in gradual stages A closer study of the figures of opium consumption district by district given in Table III shows that the reduction is not uniform In Lahore, Shahpur, Dera Gazi Khan and four other districts there is an increase while in all other districts the consumption has decreased In Lahore Shahpur and Dera Gazi Khan the rise is probably due to the agriculturists now buying opium from licensed vendors whereas previously they used to grow poppy and keep some opium for their own use In all other districts there is a reduction four districts showing a decrease of over 50 per cent, eight showing a decrease above the average of 31 per cent and the remaining eight districts (excluding Lyallpur and Sheikhupura) showing a reduction below the average (between 5 5 to 23 per cent) The districts which show the highest consumption figures also show a comparative reduction, e g, Ferozepur 31 8 per cent Ludhiana and Amritsar 11 5 per cent and 30 0 per cent respectively except Lahore which shows an increase of 6 5 per cent From these figures it would appear that the measures adopted have attained a great degree of success The limit of restrictive measures, however, seems to have been reached, and any further increase of price will defeat its own object, because the high excise duty, which increases the sale price and increases the difficulties in the way of the people procuring the drug, stimulates smuggling From our work in the field we are convinced that the limit has been reached, for the present, at any rate, in that direction, and that further measures for reducing consumption will have more chance of success if directed towards compulsory registration of addicts, etc

From the above discussion it is clear that the excise statistics help only to a limited extent to gauge the real extent of opium addiction in the Punjab We are inclined to believe that if the measures adopted have not resulted in materially reducing the number of addicts, they have certainly resulted in the addicts consuming less quantity of opium daily than in former days From the analysis of cases appended to the report of the Royal Commission on Opium of 1895, the average dose was calculated at 18 grains per addict per day The average dose in our series of 1,070 cases works out to be about 13 grains a day Although it is said that some of the addicts used to take as much as 1 to 3 tolas (i e , 180 grains to 540 grains) a day, during the course of our investigation we have not met anyone taking more than 90 grains a day The addicts themselves admit that they have to reduce their daily dose owing to the enormous increase in the price of opium Whereas 180 grains (one tola) of opium cost the consumer 8 annas in 1910-11, he had to pay three times as much for that amount in 1929-30 Besides, the cost of other

necessities of life has also gone up considerably, whilst the wage-earning capacity of the people generally has not increased correspondingly Having regard to the figures of the average dose as given in the report of the Royal Commission on Opium and the average dose worked by us it is possible that the reduction in the daily dose can fully account for 31 per cent reduction in the total quantity of opium consumed in the province The presumption, therefore is that the total number of addicts in the province has not decreased to any appreciable extent It certainly has not increased, though it must be admitted that in certain localities, especially in large towns, addiction to opium among young neurotic individuals has shown a remarkable increase

We have also tried to ascertain the number of addicts in a locality by counting them This is a very difficult procedure because the persons taking opium and their friends are often not anxious to give the information, and a certain number of addicts keep the habit secret even from their relations and friends In localities where there is a great deal of smuggling, opium eaters generally keep the habit secret for fear of being prosecuted Their friends also withhold information even when they know certain persons are opium addicts It is said that anybody can detect the so-called ' dope fiend ', but we know from our extensive experience with opium addicts in India that it is impossible to detect a person taking opium in small or in moderate quantities even after a careful physical examination It is only when very large doses are taken that the addicts show obvious subjective and objective symptoms of intoxication

The distribution of opium habit in different localities is very variable In certain villages a large number of addicts can be found, whilst in others there are few It seems that an addict is like a focus of infection, wherever he is, he persuades his fellow men to take the drug Our work in the field convinces us that climate or other environmental conditions, such as damp or the prevalence of malaria, do not play any great part in the formation of opium habit The chief factors concerned appear to be the instinctive desire of mankind to take drugs producing euphoria, along with peculiarities in the temperament of certain communities and races which make them more prone to take these drugs than others living side by side with them

As regards the method of calculating the number of addicts from the statistics of opium consumption issued by the excise departments, on the basis of average daily dose of an addict, we have pointed out the factors that vitiate these figures There is no doubt, however, that calculations based on these statistics would give us the minimal figures of the addict population We have collected 1,070 cases, which may be regarded as a fair sample, and have worked out the average daily dose at 13 grains a day per addict There also a small source of error enters into the calculations, namely, the allowance which must be made for the opium used for purely medicinal purposes in contra-distinction to that used for addiction

According to the League of Nations' Standard the estimate of opium used for medical and scientific purposes in a locality, where medical aid is efficient is 12 pounds or 6 seers per 10,000 of population per annum (0 06 seer per 100) This standard, though it can be taken as a rough guide for these calculations, is said to be unsatisfactory for a country like India where the standard of medical relief is comparatively low It has been pointed out already that ordinary excise opium is used to a very limited extent by the medical profession and practitioners of the indigenous systems, it is also used to a limited extent as a household remedy It is impossible to determine with any degree of accuracy the amount used by itinerant quacks During the second session of the Geneva Conference, in the first mixed meeting of the sub-committee of the Health Committee and the Advisory Committee on the Traffic of Opium, whilst discussing the legitimate annual medical requirements of each country, Mr (now Sir John) Campbell, the delegate for India, thought 15 grains per head per year (or roughly 0 1 seer per 100) might be fixed as the maximum requirement for India He agreed that it erred on the side of excess, as under the existing conditions the facilities for medical aid were not as adequate as in other civilized countries a safe figure was required

In this connection it may be pointed out that our work in the Punjab and other parts of India convinces us, that the facilities for medical aid existing in an area, have little or no bearing on the amount of opium consumed by the population We have no doubt that the estimate formed by the League of Nations of 0 06 seer of opium per 100 as being essential for the medical and scientific needs of the people, is applicable to the Punjab just as much as to other civilized countries with a more adequate system of medical relief In support of this it may be pointed out that there are many districts in the Punjab with lower than the average standard of medical relief in this country where the consumption of opium is less than 12 pounds or 6 seers per 10,000 of population per annum (the standard laid down by the League)

In attempting to determine the minimum number of addicts in a district we have taken the maximum figure of 0 1 seer per 100 as the amount consumed for medical and scientific purposes From the data thus obtained the number of addicts in Ferozepur district would be 1 54 per cent of the total population It is well known that a very small number of females take opium, and addiction among those under 15 years of age is very uncommon The percentage of addicts in the adult male population would thus work out to be about 3 5 per cent If we calculate in the same way the number of addicts for Moga tahsil, where the Sikh population is the highest in the province, the percentage of opium addicts is probably twice as much, i e , 7 per cent of the adult male population Calculations on this basis have been made, but these must necessarily be verified by extensive field work, which is not possible with the staff and funds

at our disposal The accuracy of our information on this particular point is not confirmed

Summary and conclusions

1 Before the British rule was established in the Punjab, poppy was grown in almost every part of the province without restriction Since the British administration has been organized poppy cultivation and opium production has progressively reduced At first the cultivation of poppy was allowed only under special license In 1897 the cultivation was stopped by notification under the Opium Act of 1878, exceptions being made only in cases of certain districts where it formed a staple crop on which the cultivator depended for his livelihood In 1907 the cultivation was confined to small tracts, (1) for the production of 'home grown' opium and (2) for the production of 'post' or poppy heads In 1909 the cultivation for production of opium was altogether prohibited in the plain districts, a very limited area for the production of poppy heads was allowed in Jullundur and Hoshiarpur districts Total suppression is now in contemplation

2 The Punjab used to be self-supporting both as regards its supply of opium and of poppy heads Owing to the stoppage of cultivation of the poppy most of the supply now comes from the Government of India factory at Ghazipur This affords a better control

3 The use of opium for medicinal purposes in the province is very limited The medical practitioners use opium and its derivatives to a much smaller extent than their confreres in the Western countries Opium is very little used by the practitioners of indigenous medicine, though itinerant quacks use it fairly frequently Opium has never been used as a prophylactic against, or in the treatment of, malaria in this part It is used to a limited extent to control intestinal fluxes, colds, coughs, etc Its use for administration to domestic animals is very limited at the present time owing to its high price

4 The excessive use of opium in the province has no relation whatsoever to the insufficiency of medical relief Most of the raw opium sold is consumed by the addicts

5 The average consumption in the Punjab works out to be 0 18 seer per 100 of population per year (as compared with 0 06 seer per 100 which, according to the League of Nations, is the amount necessary for the medical and scientific needs of the population) The excessive consumption is due to certain communities taking the drug habitually By far the largest majority of the population consume even less than the standard amount laid down by the League The addiction is not widespread, but is confined to certain communities and certain areas The problem is therefore not serious when the province is considered as a whole

6 The rise in opium consumption runs almost parallel with the increase in the percentage of the Sikhs among the population The areas with a preponderance

of the Mohammedans and a large Hindu population consume very little opium In the towns opium habit is to be found among all communities, but more so among the Hindus and the Sikhs

7 The reason why the Sikhs are more addicted to the use of opium is not clear Probably the fact that they are a martial race who have been subjected to many vicissitudes of fortune and have undergone much physical strain and stress during the past century and a half may be responsible The Rajputs who were similarly situated are also addicted to opium, but many of the other martial races of India appear to show no such tendency

8 The opium consumption in the Punjab decreased by 31 per cent in 1929-30 as compared with 1900-01 This decrease is undoubtedly due to restrictive measures adopted by the authorities The decrease may be either (1) decrease in the number of addicts, or (2) decrease in the quantity taken by the addicts owing to its high price The average dose per man per day was worked out at 18 grains by the Opium Commission of 1895, while in our series of 1,070 cases it is only 13 grains daily The whole of the decrease in the amount consumed can be accounted for by this decrease in the dosage

9 Whatever may be the case, it is certain that opium addiction is not on the increase, but has appreciably decreased during the last 20 years

10 Our work in the Punjab convinces us that climatic and other environmental factors, such as cold and damp, prevalence of malaria, etc, do not play any great part in leading the people to form the habit of eating opium

11 The chief factors concerned appear to be the instinctive desire of mankind to take drugs which produce euphoria, and also the racial predilection which makes certain races and communities living side by side temperamentally more prone than others to take to such drugs

12 The standard laid down by the League of Nations regarding the quantity of opium necessary for the medical and scientific needs of the population holds good in the case of the Punjab

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SOME FURTHER RECORDS OF *PHLEBOTOMUS* FROM AFRICA

BY

LIEUT.-COLONEL J A SINTON, M D, D SC, I M S

(*From the Malaria Survey of India Kasauli*)

[Received for publication, May 31, 1932]

SINCE my previous paper on the sandflies of Africa (Sinton, 1930), collections of these insects have been sent to me from Mombasa, Kenya and Transvaal, through the kindness of Dr C B Symes, Dr I I Roberts and Dr B de Meillon respectively, to all of whom I wish to express my sincere thanks

PHLEBOTOMUS FROM TRANSVAAL

Dr de Meillon has sent a collection of *Phlebotomus*, labelled as follows 'Phlebotomus, Letsitelle, Tzaneen, Tvl, South Afr 1-3-32 (Taken in native hut)'

This collection contained the following insects —

Phlebotomus schwetzi ADLER, THEODOR AND PARROT, 1929

Theodor (1931) has pointed out that the species described by Sinton (1930) from East Africa as *P symesi* is identical with that previously described by Adler, Theodor and Parrot (1929) from the Belgian Congo

Six females and 3 males of this species were included in the collection from Transvaal

Phlebotomus meilloni SP N

Four females and seven males of a new species of *Phlebotomus* were received from Letsitelle These insects belong to the group with scanty erect hairs on the dorsal surfaces of abdominal segments II–VI, i e, the sub-genus *Sintonius* of Nitzulescu (1931) No other species of this sub-genus have been described from Africa, and it is proposed that this one should be named *Phlebotomus meilloni* after its discoverer

Phlebotomus meilloni (♀)

The specimens were not differentiated in the dry state from *P schwetzi* with which they were mixed in the collection

Appearances in stained and mounted specimens

The measurements of the type and three paratype females are given in Table I

The *total length* of the insect is about 3 0 mm The cicatrices of a few erect and many recumbent hairs are present on the dorsum of the abdomen

The *buccal cavity* (Plate XVIII, fig 2) shows a well-developed mushroom-shaped pigmented area The buccal teeth are large and pointed, they are arranged in a single, almost straight, row with a slight posterior curve near the middle line They number about 16

The *pharynx* (Plate XVIII, fig 3) has an armature of minute spines in groups arising from small transverse curved ridges The spines on the posterior ridges are longer than those on the anterior The greatest width of the pharynx posteriorly is about twice that of its narrow anterior portion and its length is about $2\frac{1}{2}$ times its greatest breadth

TABLE I

Phlebotomus mellon (♀ ♀) SP N

	Structures	Lengths in mms of specimens number —				Remarks, relative lengths, etc
		1*	2†	3†	4†	
Body	Head and clypeus	0 430	0 470	0 400	0 430	
	Thorax	0 685	0 770	0 714	0 757	
	Abdomen proper	1 530	1 714	1 657	1 685	
	Sup clasper	0 170	0 143	0 157	0 170	
	Total length	2 81	3 1	2 93	3 04	
	Labium	0 257	0 285	0 270	0 270	
	Pharynx, length	0 170	0 183	0 171	0 168	= 2 33—2 53 × breadth
	Pharynx, breadth	0 067	0 075	0 067	0 072	
Antenna	Segment III	0 300	0 310	0 312	0 290	= 0 75 × XII—XVIth
	Segment IV	0 141	0 147		0 141	IV + V < IIIrd
	Segment V	0 138	0 138		0 130	Formula = $\frac{2}{\text{III—XV}}$
	Segment VI	0 132	0 135		0 126	
	Segments XII–XVI	0 402	0 408		0 384	
	Total length	1 860	1 885		1 785	= 6 2 × IIIrd, 4 66 × XII—XVIth

* Type female. † Paratype females

TABLE I—*concld*

	Structures	Lengths in mms of specimens number — 1*	2†	3†	4†	Remarks, relative lengths, etc
Palp	Segment 1	0 048	0 045	0 048	0 045	Relative lengths 2 2, 5 7, 8 2, 10, 17 5
	Segment 2	0 120	0 126	0 120	0 120	$1 + 2 \leqq 3$
	Segment 3	0 174	0 186	0 174	0 174	Formula 1, 2, 3, 4, 5
	Segment 4	0 213	0 228	0 210	0 210	
	Segment 5	0 360	0 405	0 360	0 372	
	Total length	0 915	0 990	0 912	0 921	
Wing	Length	2 170	2 300	2 143	2 300	= 3 3—3 4 × breadth
	Breadth	0 643	0 685	0 643	0 657	
	a	0 557	0 585	0 543	0 557	$\frac{a}{\beta} = 1\ 66—1\ 81\ \frac{\beta}{\gamma} = 0\ 90—0\ 95$
	β	0 307	0 320	0 320	0 335	$\frac{a}{\gamma} = 1\ 56—1\ 69\ \frac{\delta}{a} = 0\ 66—0\ 69$
	γ	0 330	0 357	0 330	0 357	
	δ	0 385	0 385	0 357	0 370	$\frac{a}{\varepsilon} = 0\ 81 \quad \frac{\theta}{\varepsilon} = 1\ 60—1\ 65$
	ε	0 685	0 714	0 670	0 613	
	θ	1 093	1 143	1 100	1 143	$\frac{a+\beta}{\theta} = 0\ 78 \quad \frac{\text{Wing}}{\theta} = 1\ 95—2\ 00$
Hind leg	Femur	0 843	0 943	0 857	0 900	$< \frac{1}{4} \times$ leg
	Tibia	1 307	1 443	1 330	1 357	= 1 55 × femur, 1 75–1 8 × tarsus, seg 1
	Tarsus, seg 1	0 720	0 827	0 757		
	Tarsus, segs 2–5	0 757	0 814	0 785		$\geqq$ 1 5th leg
	Total length	3 63	4 04	3 73		(Not including coxa and trochanter)
	Spermatheca, length	0 043	0 042	0 036	0 042	= 3 × breadth
	Spermatheca, breadth	0 013	0 014	0 013	0 013	

* Type female † Paratype females

The *antenna* (Plate XVIII, figs 4 to 6) have paired geniculate spines on segments III to XV, these are comparatively short Segment III is very long (about 0 3 mm) and reaches slightly beyond the end of the proboscis This segment is longer than the combined length of segments IV and V, but only

about three-fourths that of segments XII–XVI The terminal segment is distinctly elongated

The *palp* (Plate XVIII, fig 10) has a formula of 1, 2, 3, 4, 5 and the relative lengths of the segments in 4 specimens averaged 2 2, 5 7, 8, 2, 10, 17 5 The combined length of segments 1 and 2 is slightly less than that of 3 Newstead's spines are situated on the basal third of segment 3 and are about 10–12 in number

The *wing* (Plate XVIII, fig 1) is broadly lanceolate and about 3 4 times as long as broad β is about equal to γ, while both these lengths are much smaller than α, the ratio δ over α is about 0 66

The *hind leg* is about 5 times the length of tarsal segment 1 and is much longer than the wing The femur forms about 0 23 of the leg length, the tibia is about $1\frac{1}{2}$ times the length of the femur and $1\frac{3}{4}$ times that of tarsal segment 1

The *spermatheca* (Plate XVIII, fig 7) is sausage-shaped and is about 3 times as long as broad, it shows marked crenulation into 10 segments and the head is small The spermathecal ducts (Plate XVIII, fig 8) are very long, being about 8 times the length of spermatheca, these ducts unite into a common tube close to the vulva

Phlebotomus mellon (♂)

The measurement of the type and three paratype males are given in Table II The relative lengths were determined from the seven specimens available

TABLE II

Phlebotomus mellon (♂♂) N SP

Structures		Lengths in mms of specimens number —				Remarks, relative lengths, etc
		1	2	3	4	
Body	Clypeus and head	0 357	0 400	0 400	0 430	
	Thorax	0 585	0 643	0 714	0 743	
	Abdomen proper	1 428	1 643	1 643	1 930	
	Sup clasper, seg 1	0 270	0 294	0 315	0 315	
	Total length	2 64	2 98	3 07	3 4	
	Labium	0 257	0 264	0 270	0 285	
	Pharynx, length	0 150	0 165	0 180	0 180	=2 4—2 8 × breadth
	Pharynx, breadth	0 063	0 057	0 066	0 063	

TABLE II—*contd*

Structures		Lengths in mms of specimens number — 1	2	3	4	Remarks, relative lengths, etc
Antenna	Segment III	0 360	0 366	0 405	0 400	=0 83—0 90× XII–XVI
	Segment IV	0 177	0 186	0 210	0 200	IV + V ≅ III
	Segment V	0 165	0 171	0 192	0 190	Formula $\frac{1}{III-XV}$
	Segment VI	0 156	0 165	0 183	0 180	
	Segments XII–XVI	0 405	0 426	0 480	0 465	
	Total length	2 070	2 170	2 400	2 330	=5 75—6 0× IIIrd, 5 0—5 28×XII–XVI
Palp	Segment 1	0 048	0 051	0 051	0 051	Relative lengths 2 1, 5 8, 8 2, 10, 17 9
	Segment 2	0 126	0 135	0 141	0 138	1 + 2 ≅ 3
	Segment 3	0 174	0 190	0 195	0 198	
	Segment 4	0 213	0 228	0 240	0 240	Formula 1, 2, 3, 4, 5
	Segment 5	0 375	0 402	0 423	0 426	
	Total length	0 936	1 006	1 050	1 053	
Wing	Length	2 000	2 214	2 343	2 400	=3 6—3 8× breadth
	Breadth	0 520	0 585	0 650	0 664	$\frac{\alpha}{\beta}$=1 26—1 66 $\frac{\beta}{\gamma}$=0 83—1 17
	α	0 457	0 500	0 570	0 600	
	β	0 340	0 385	0 385	0 370	$\frac{\alpha}{\gamma}$=1 36—1 52 $\frac{\delta}{\alpha}$=0 60—0 73
	γ	0 328	0 328	0 370	0 428	
	δ	0 285	0 314	0 357	0 380	$\frac{\alpha}{\epsilon}$=0 77—0 80 $\frac{\theta}{\epsilon}$=1 60—1 80
	ϵ	0 570	0 643	0 700	0 743	
	θ	1 028	1 143	1 214	1 214	$\frac{\alpha+\beta}{\theta}$=0 76—0 78 $\frac{\text{Wing}}{\theta}$=1 90—1 97
Hind leg	Femur	0 800	0 900	0 970	0 943	=0 22× length leg
	Tibia	1 300	1 457	1 585	1 543	=1 62–1 67×femur, 1 72–1 76× tarsus, seg 1
	Tarsus, seg 1	0 757	0 828	0 914	0 885	
	Tarsus, segs 2–5	0 714	0 785	0 857	0 843	= 1 5th leg
	Total length	3 57	3 97	4 33	4 2	(Not including coxa and trochanter)

TABLE II—*concld*

	Structures	Lengths in mms of specimens number —				Remarks, relative lengths, etc
		1	2	3	4	
Genitalia	Sup clasper, seg 1	0 270	0 294	0 315	0 315	=2 18×seg 1, 1 10×inf clasper
	Sup clasper, seg 2	0 123	0 135	0 144	0 144	
	Intermed append	0 210	0 225	0 250	0 255	=0 76-0 81× seg 1
	Intromittent organ	0 174	0 190	0 210	0 210	=0 80-0 84×intermed append
	Genital filament	0 105	0 090	?	0 108	(Length protruded)
	Inferior clasper	0 240	0 270	0 280	0 280	=1 30-1 35× subgen lamella
	Subgen lamella	0 180	0 204	0 213	0 216	

The *total length* averaged about 2 9 mm The cicatrices of a few erect hairs are visible on the dorsal surfaces of segments II–IV of the abdomen

The *buccal cavity* (Plate XIX, fig 13) has a small but distinct turnip-shaped pigmented area The buccal teeth are about 10 in number, they are wider than in the female and in many instances show serrated ends The *pharynx* (Plate XIX, fig 14) resembles that of the female but is less markedly dilated posteriorly, the armature is less well developed

The *antenna* (Plate XIX, figs 15 to 17) has a unilateral spine on each of segments III to XV, these are comparatively short Segment III is very long, in some cases 0 4 mm, and reaches well beyond the tip of the proboscis This segment is about the same length as segments IV and V combined

The *palp* (Plate XIX, fig 12) has a formula of 1, 2, 3, 4, 5 and the relative lengths of the segments averaged 2 1, 5 8, 8 2, 10, 17 9 Newstead's spines are situated on the basal third of the 3rd segment and are about 6 in number

The *wing* (Plate XIX, fig 11) is relatively narrower and more pointed than in the female, being about 3 7 times as long as broad β is almost equal to γ, while each of these lengths is only about half that of α, the ratio δ over α is about 0 64

The *hind leg* is about 5 times the combined length of tarsal segments 2 to 5 and is much longer than the wing The femur forms about 0 22 of the leg

The *male genitalia* (Plate XIX, fig 19) are of the *minutus* type The distal segment of the superior clasper bears 4 large curved spines with spatulate ends

Two of these are apical and two sub-apical, they are nearly as long as the segment which bears them The small non-deciduous spine arises near the level of the sub-apical spines (Plate XIX, fig 20) The intromittent organ (Plate XIX, fig 18) has a pointed end and in five of the seven specimens the genital filament is markedly protruded, this filament has a truncated end The pompetta lies about the 6th abdominal segment

Differential diagnosis of P meilloni

So far no species of the sub-genus *Sintonus* have been described from Africa, but in Asia it is represented by *P hospitii*, *P christophersi*, *P clydei* and *P tiberiadis* The morphology of the buccal and pharyngeal armatures at once differentiates *P meilloni* from these species In addition —

(*a*) *P hospitii* Sinton, 1924, has a similar palpal formula but segment 2 is relatively shorter, none of the spines on the distal segment of the superior clasper are markedly sub-apical and the non-deciduous spine is markedly proximal to these spines

(*b*) *P christophersi* Sinton, 1927, has a much shorter IIIrd antennal segment, the end of which does not nearly reach the tip of the proboscis, the palpal formula is 1, 2, 3, 4, 5, the wing has a different venation and its length is more than 4 times its breadth, the inferior clasper is about equal in length to segment 1 of the superior clasper and the non-deciduous spine is distinctly proximal to the other spines of the superior clasper, which are grouped more apically

(*c*) *Phlebotomus clydei* Sinton, 1928, has a much shorter IIIrd antennal segment, which does not reach to the end of the proboscis, the palpal formula is 1, 2, 4, 3, 5, the wing has a different venation and is about 4 times as long as broad, the inferior clasper is about equal to segment 1 of the superior clasper and the non-deciduous spine is distinctly proximal to the other spines of the superior clasper, which are grouped more apically

(*d*) *Phlebotomus tiberiadis* Adler, Theodor and Lourie, 1930 This species was described by Adler and Theodor (1929) as '*Phlebotomus* sp near *clydei* Sinton' It is only 1 5–1 8 mm long, the palpal formula is 1 2, 4, 3, 5, α is much shorter than β, δ is very small, the pigmented area is heart-shaped and very faint, the pharynx is unarmed, and the spermathecal ducts end separately

Phlebotomus africanus VAR **magnus** NOV VAR

One female specimen resembling *P africanus* Newstead was found in the collection from Letsitelle Unfortunately the antennæ and most of the legs were missing The measurements of the remaining portions are given in Table III

TABLE III

Phlebotomus africanus VAR **magnus** (♀) NOV VAR

	Structures	Length in mms	Remarks, relative lengths, etc
Body	Clypeus and head	0 400	
	Thorax	0 630	
	Abdomen proper	1 285	
	Superior clasper	0 143	
	Total length	2 45	
	Labium	0 200	
	Pharynx, length	0 160	= 2 96 × breadth
	Pharynx, breadth	0 054	= 2 4 × narrowest portion
Palp	Segment 1	0 040	Formula 1, 2, 3, 4, 5
	Segment 2	0 080	Relative lengths 2 5, 5 0, 8 7, 10, 19 8
	Segment 3	0 140	1+2 << 3
	Segment 4	0 160	
	Segment 5	0 318	3rd seg incrassate N's spines very numerous
	Total length	0 738	
Wing	Length	1 770	3 7 × breadth, 2 5 × femur
	Breadth	0 485	
	α	0 270	$\frac{\alpha}{\beta} = 0\,79$ $\frac{\beta}{\gamma} = 1\,41$ $\frac{\alpha}{\gamma} = 1\,11$
	β	0 343	
	γ	0 243	$\frac{\delta}{\alpha} = 0\,42$ $\frac{\alpha}{\varepsilon} = 0\,63$ $\frac{\theta}{\varepsilon} = 2\,06$
	δ	0 114	
	ε	0 428	$\frac{\alpha+\beta}{\theta} = 0\,69$ $\frac{\text{Wing}}{\theta} = 2\,0$
	θ	0 885	
	Femur	0 714	
	Spermatheca, length	0 066	= 2 75 × breadth.
	Spermatheca, breadth	0 024	

This specimen differs in several particulars from the descriptions of *P africanus* from Palestine given by Adler and Theodor (1926 and 1927) and by Sinton (1930), and more closely resembles that given by Adler, Theodor and Parrot (1929) for specimens from the Belgian Congo It seems worthy of varietal rank and it is proposed that it be called *P africanus* var *magnus*

From the measurements it will be seen that the variety more closely resembles *P freetownensis* (Sinton, 1930) than *P africanus*, but the buccal teeth are only about 40 in number as in the latter species and the pharyngeal armature is very similar (Plate XX, figs 32 and 33) but the spines appear to be more numerous, i e , intermediate between *P africanus* and *P freetownensis*

The specimen also differs from the typical *P africanus* of Palestine and India in its larger size its broader and less lanceolate wings, the larger size of ♀ and ♂ the combined length of palpal segments 1 and 2 is very much less than that of 3, which segment is distinctly shorter than 4 and the spermatheca is relatively longer and narrower being about $2\frac{1}{4}$ times as long as broad

PHLEBOTOMUS FROM MOMBASA

Three collections of sandflies were received from Dr C B Symes from Mombasa, in which were found the following species —

Phlebotomus schwetzi ADLER, THEODOR AND PARROT, 1929

(*a*) 'March, 1930'—35 ♂♂, 117 ♀♀ (*b*) 'Houses, 3-6-30'—7 ♂♂, 29 ♀♀ (*c*) 'Prison, 24-4-30'—5 ♂♂, 22 ♀♀

Phlebotomus yusafi SINTON, 1930

(*a*) 'March, 1930'—2 ♀♀ (*b*) 'Houses, 3-6-30'—4 ♀♀, 3 ♂♂ (*c*) 'Prison, 24-4-30'—1 ♂

Phlebotomus africanus NEWSTEAD, 1912

(*a*) 'March, 1930'—1 ♂ (*b*) 'Prison, 24-4-30'—1 ♀

Phlebotomus meillonı VAR **suberectus** NOV VAR

In each of the collections 'March, 1930' and 'Prison', a single male belonging to the sub-genus *Sintonus* were found These specimens resemble *P meillonı* but differ in several important particulars These insects have been placed provisionally as a variety of *P meillonı*, but it seems probable that, when the females are found, it will be seen to be a distinct species

The measurements of the two specimens are given in Table IV and drawings of the different parts in Plate XX, figs 21 to 30

TABLE IV

Phlebotomus mellloni VAR **suberectus** (♂ ♂) NOV VAR

	Structures	Length in mms of specimens number — 1*	2†	Remarks, relative lengths, etc
	Clypeus and head	0 500	0 430	
	Thorax	0 714	0 685	
	Abdomen proper	1 670	1 757	
	Sup clasper, seg 1	0 321	0 300	
	Total length	3 2	3 2	
	Labium	0 257	0 257	
	Pharynx, length	0 165	0 165	= 2 5 × breadth
	Pharynx, breadth	0 066		
Antenna	Segment III	0 360	0 340	= 0 83 × XII–XVI
	Segment IV	0 165	0 160	IV + V < III
	Segment V	0 156	0 153	Formula $\frac{1}{\text{III–XV}}$
	Segment VI	0 153	0 144	
	Segments XII–XVI	0 430		
	Total length	2 110		= 5 8 × IIIrd, 5 0 × XII–XVIth
Palp	Segment 1	0 054	0 054	Relative lengths 2 0, 4 7, 7 2, 10, 17 8
	Segment 2	0 126	0 126	1 + 2 < 3
	Segment 3	0 192	0 192	
	Segment 4	0 270	0 261	Formula 1, 2, 3, 4, 5
	Segment 5	0 504	0 444	
	Total length	1 146	1 077	

* Type male

† Paratype male

TABLE IV—*concld*

	Structures	Length in mms of specimens number — 1*	2†	Remarks, relative lengths, etc
Wing	Length	2 260	2 257	= 3 33 × breadth
	Breadth	0 670	0 680	$\frac{a}{\beta}$ = 1 86–2 1 $\frac{\beta}{\gamma}$ = 0 88–0 91
	a	0 585	0 614	
	β	0 314	0 300	$\frac{a}{\gamma}$ = 1 64–1 87 $\frac{\delta}{a}$ = 0 69–0 72
	γ	0 357	0 325	
	δ	0 400	0 443	$\frac{a}{\varepsilon}$ = 0 82 $\frac{\theta}{\varepsilon}$ = 1 57–1 64
	ε	0 714	0 743	
	θ	1 170	1 170	$\frac{a+\beta}{\theta}$ = 0 77 $\frac{\text{Wing}}{\theta}$ = 1 92
Hind leg	Femur	0 930	0 885	
	Tibia	1 500	1 370	= 1 60 × femur, 1 7 × tarsus, seg 1
	Tarsus, seg 1		0 800	
	Tarsus, segs 2–5		0 814	> 1 5th leg
	Total length		3 87	
Genitalia	Sup clasper, seg 1	0 321	0 306	= 2 15 × seg 2, 1 06 × inf clasper
	Sup clasper, seg 2	0 150	0 141	< ½ seg 1
	Intermed append	0 246	0 246	= 0 77–0 80 × seg 1
	Intromittent organ	0 195	0 200	= 0 80 × intermed append
	Genital filament	0 330	0 136	(distance protruded)
	Inferior clasper	0 300	0 290	= 1 47 × subgen lamella, 1 18–1 22 × intermed append
	Subgen lamella	0 204	0 198	

* Type male † Paratype male

Differential diagnosis

This variety differs from *P meillonı* type in the following characters — (*a*) The buccal teeth, number about 22–25, are much smaller and the row shows a distinct concavity backwards (*b*) The pharynx is more dilated posteriorly (*c*) The non-deciduous spine is distinctly proximal to the other spines on the distal segment of the superior clasper, and of these the sub-apical pair are more proximal than in the type (*d*) Segments 2 and 3 of the palp are relatively shorter (*e*) The antennal spines are relatively longer (*f*) The wing is relatively wider (*g*) The ratio a over β is greater.

Phlebotomus from Kenya

Dr Roberts sent a collection of 5 males and 7 females *Phlebotomus* labelled 'Taveta, Kenya About 2,000 ft 28-10-30 Biting viciously'

These specimens all belonged to the species described by Theodor (1931) as *Phlebotomus nairobiensis*

It is interesting to note that Theodor (1931) says 'only two species of the *minutus* group have been observed so far to bite man, *P schwetzi* (Schwetz, 1928) and *P babu* (Lloyd and Napier 1930)' *P nairobiensis* must therefore be added to the list of human pests

Summary

Descriptions are given of one new species of *Phlebotomus* (*P meillon*) and two new varieties (*P meillon* var *suberectus* and *P africanus* var *magnus*) from Africa Fresh records of the African distribution of *P schwetzi*, *P nairobiensis*, *P yusafi* and *P africanus* are also noted

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Idem	(1928)	*Ibid*, **16**, 1, pp 179–186
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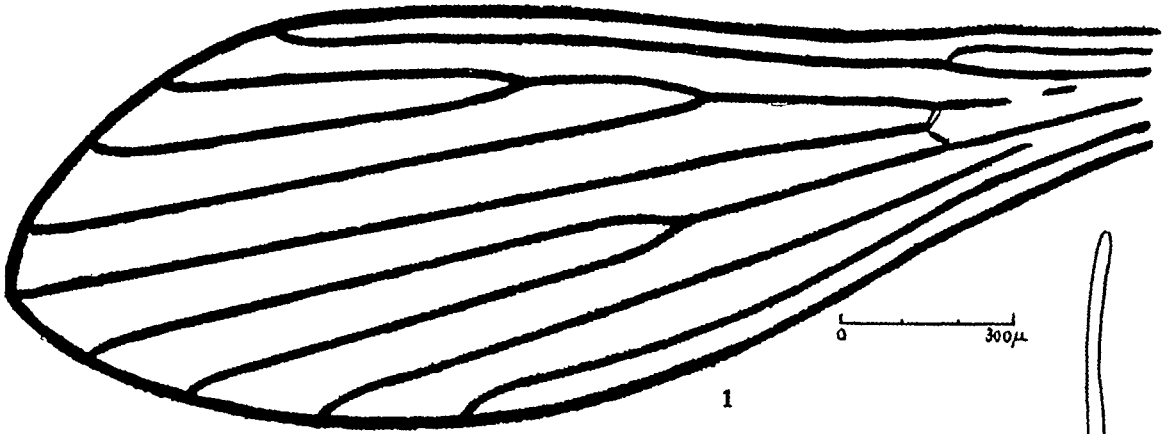
Theodor, O	(1931)	*Bull Entom Res*, **22**, 4, pp 469–478

Explanation of Plate XVIII

Phlebotomus meillon (♀)

Fig 1 Wing
„ 2 Buccal cavity
„ 3 Pharynx
„ 4 Antenna, segment III
„ 5 Antenna, segment IV
„ 6 Antenna, segments XIV–XVI
„ 7 Spermatheca
„ 8 Spermatheca and spermathecal ducts
„ 9 Furca
„ 10 Palp N—Newstead's spines.

PLATE XVIII



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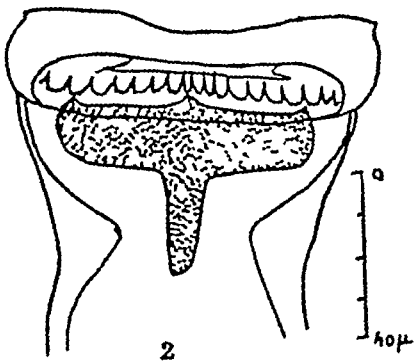

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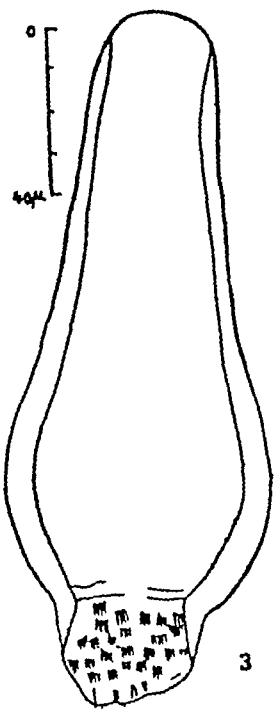

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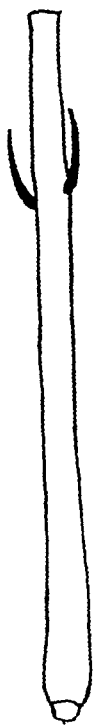

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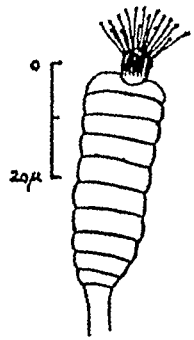

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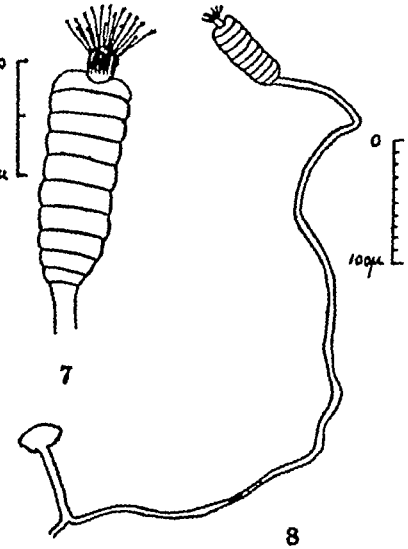

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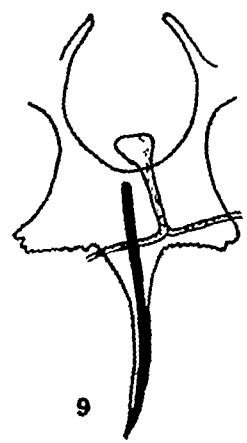

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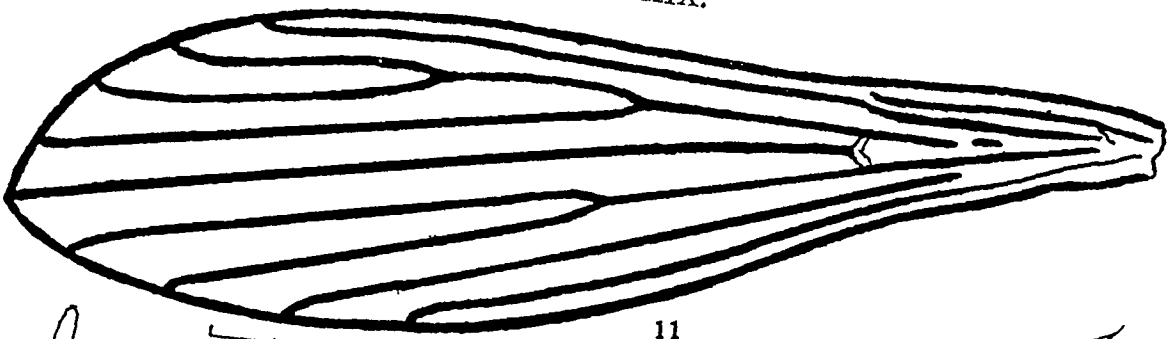
Explanation of Plate XIX

Phlebotomus meillon (♂)

Fig 11. Wing
„ 12 Palp N —Newstead's spines
„ 13 Buccal cavity
„ 14 Pharynx
„ 15. Antenna, segment III
„ 16 Antenna, segment IV
„ 17 Antenna, segments XIV–XVI
„ 18 Intromittent organ
„ 19 Male genitalia
„ 20 Distal segment of superior clasper

PLATE XIX.



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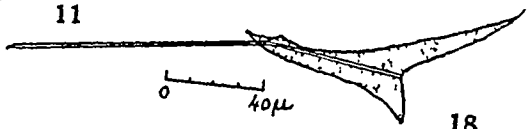

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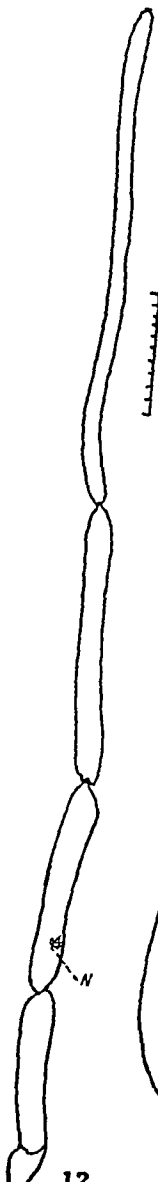

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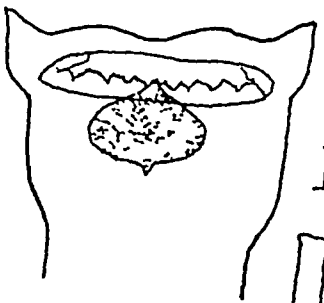

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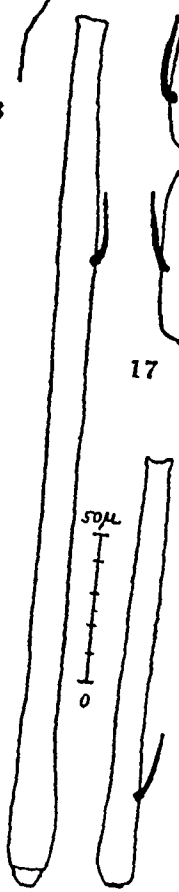

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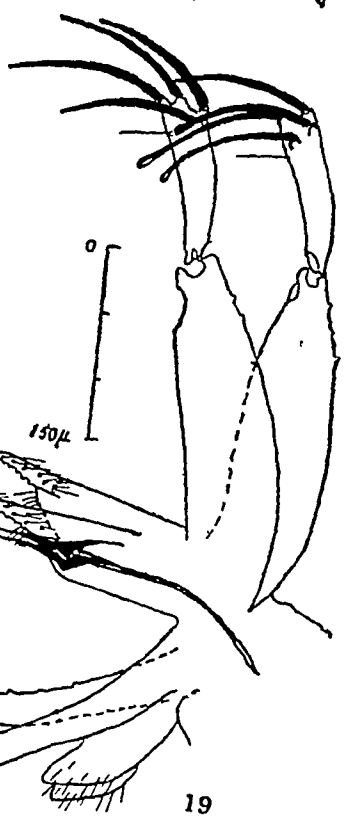

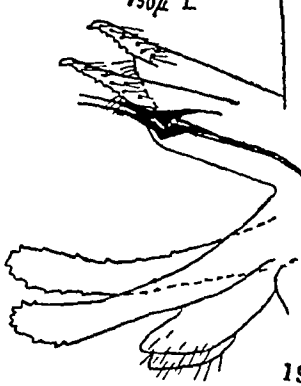

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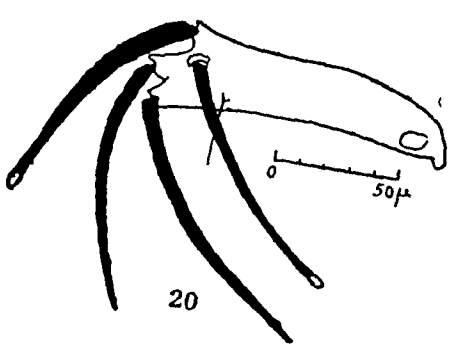

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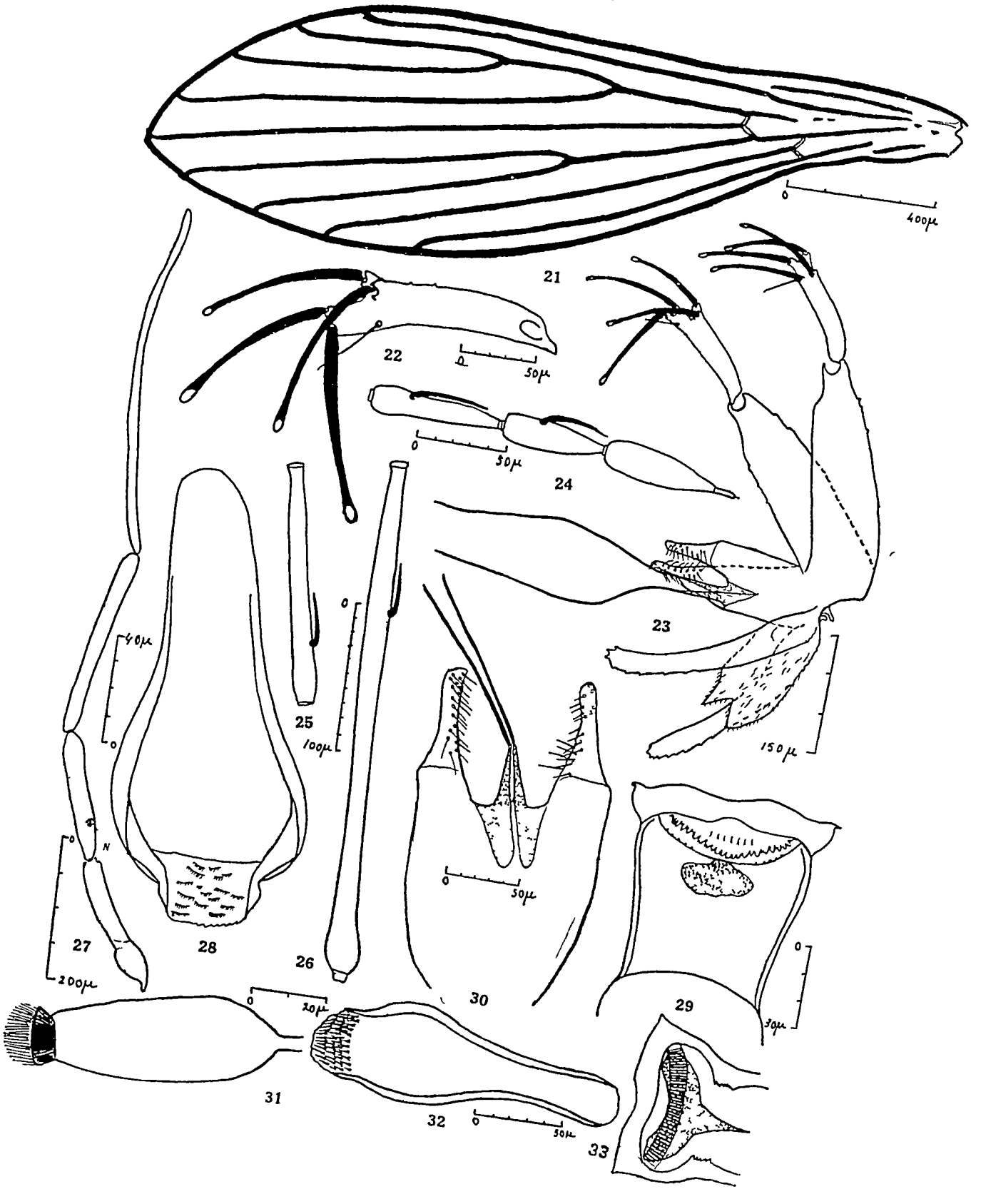
Explanation of Plate XX

Phlebotomus mesllom var *suberectus* (♂) (Figs 21 to 30) and *Phlebotomus africanus* var *magnus* (♀) (Figs 31 to 33)

Fig 21 Wing
" 22 Distal segment of superior clasper.
" 23 Male genitalia
" 24 Antenna, segments XIV–XVI
" 25 Antenna, segment IV.
" 26 Antenna, segment III
" 27 Palp N—Newstead's spines
" 28 Pharynx
" 29 Buccal cavity.
" 30 Intermediate appendages and intromittent organ from above
" 31 Spermatheca
" 32 Pharynx
" 33 Buccal cavity

PLATE XX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NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

Part XXXI

PHLEBOTOMUS EADITHÆ N SP

BY

LIEUT-COLONEL J A SINTON, M D, D SC, I M S

(*From the Malaria Survey of India, Kasauli*)

[Received for publication, June 28, 1932]

DURING 1927 two female specimens of *Phlebotomus*, collected at Saugor, Central Provinces (*c* 1,700 ft), on 3rd August, were received from the Officer-in-Charge, District Laboratory, Mhow One of these was *P papatasii*, while the other, an incomplete specimen, was labelled provisionally *P hospitii* var, pending the receipt of other specimens of the insect which seemed to be a new species As no further specimens have been received since then, it is proposed to describe this species as *Phlebotomus eadithæ*

Phlebotomus eadithæ N SP (♀)

No description of the insect is available in the dry state

Appearances in stained and mounted specimen

The measurements, etc, of the type female are given in the Table

TABLE

Phlebotomus eadithæ (♀) N SP

	Structures	Length in mm	Remarks, relative lengths, etc
Body	Clypeus and head	0 430	
	Thorax	0 643	
	Abdomen proper	1 285	
	Superior clasper	0 143	
	Total length	2 50	

TABLE—*concld*

	Structures	Length in mm	Remarks, relative lengths, etc
	Labium	0 225	
	Pharynx, length	0 186	
	Pharynx, breadth	0 063	
Antenna	Segment III	0 150	III < IV + V
	Segment IV	0 088	
	Segment V	0 087	Formula = $\frac{1}{\text{III}}, \frac{2}{\text{IV-XIII}}$ (?)
	Segment VI	0 087	
	Segments XII–XVI		
Palp	Segment 1	0 036	Formula 1, 2, 3, 4, 5
	Segment 2	0 090	Relative lengths 2 3, 5 8, 9 2, 10, ?
	Segment 3	0 144	1 + 2 < 3
	Segment 4	0 156	Newstead's spines 20 or more
	Segment 5		
Wing	Length	1 857	3 82 × breadth
	Breadth	0 485	
	α	0 257	$\frac{\alpha}{\beta} = 0\ 64$ $\frac{\beta}{\gamma} = 1\ 66$ $\frac{\alpha}{\gamma} = 0\ 85$
	β	0 400	
	γ	0 300	$\frac{\delta}{\alpha} = 0\ 33$ $\frac{\theta}{\varepsilon} = 2\ 28$ $\frac{\alpha}{\varepsilon} = 0\ 64$
	δ	0 085	$\frac{\alpha+\beta}{\theta} = 0\ 72$ $\frac{\text{Wing}}{\theta} = 2\ 0?$
	ε	0 400	
	θ	0 914	
	π	0 100	
	Spermatheca, length	0 070	— 4 6 × breadth, 12 crenulations
	Spermatheca, breadth	0 015	

The *total length* of the insect is about 2 5 mm Cicatrices of both erect and recumbent hairs are present on the dorsal surfaces of abdominal segments II to VI

The *buccal cavity* (Plate XXI, fig 2) has a well-marked armature of about 35 large, pointed teeth These are arranged in a contiguous row with a marked posterior convexity near the middle line The pigmented area (Plate XXI, fig 2)

is well developed and is shaped like a low truncated cone with the apex pointing anteriorly

The *pharynx* (Plate XXI, fig 7) has a poorly developed armature of minute spines The greatest width of the pharynx posteriorly is about $2\frac{3}{4}$ times its narrow anterior portion and its length is about 3 times its greatest breadth

The *antennæ* (Plate XXI, figs 4 and 5) are unfortunately incomplete, and no segments are present beyond XIII Paired geniculate spines are present on segments IV to XIII inclusive, but there seems to be only one spine on segment III, a point which will require confirmation when other specimens are available The spines are short and stout Segment III is about 0 15 mm long

The *palp* (Plate XXI, fig 6) has segment 5 missing on both sides The formula seems to be 1, 2, 3, 4 5 and the relative lengths of the segments present are 2 8, 5 8 9 2, 10 Newstead's spines are situated on the basal third of segment 3 and number about 20 (?) Segments 1, 2 and 3 are very stout as compared with 4

The *wing* (Plate XXI, fig 1) is about 3 8 times as long as broad α is much shorter than β, which is equal to ϵ, β is much greater than γ, while α is less than γ, the ratio δ over α is about 0 33

The *spermathecæ* (Plate XXI, fig 8) is sausage-shaped and has about 12 segments It is about $4\frac{1}{2}$ times as long as broad and has a small head

Differential diagnosis —This species belongs to the group with both erect and recumbent hairs on the dorsum of the abdomen (Sinton, 1928*a*), the group which Nitzulescu (1931) has named sub-genus *Sintonius* Four species of this sub genus are recorded from Asia, namely, *P hospitii*, *P christophersi* and *P clydei* from India and *P tiberiadis* from Palestine One species *P meilloni* has been found in Africa

The following are the points of differentiation from these species from *P eadithæ* (*a*) *P hospitii* (Sinton, 1924, 1932) has over 50 buccal teeth which are arranged in an almost straight row, the IIIrd antennal segment is about 0 27 mm long and is greater than the combined lengths of segments IV and V, the relative lengths of the palpal segments is about 2 1, 6 4, 8 4, 10, 18 1, α is much greater than β, γ is much larger than β, and the ratio δ over α is about 0 66 (*b*) *P christophersi* (Sinton, 1927, 1932) has a palpal formula of 1, 2, 4, 3, 5, the pigmented area is poorly developed, the buccal teeth are few and small, β is equal to γ (*c*) *P clydei* (Sinton, 1928, 1932) has a palpal formula of 1, 2, 4, 3, 5, the pigmented area has not a truncated apex, the buccal teeth are only about 10 to 15 and β is equal to γ (*d*) *P tiberiadis* (Adler and Theodor, 1929, Adler, Theodor and Lourie, 1930) has a palpal formula of 1, 2, 4, 3, 5, it is less than 2 mm in length, the pigmented area is smaller, the buccal teeth only number about 16 and the spermatheca has only 6 to 8 segments (*e*) *P meilloni* (Sinton, 1932*a*) is about 3 mm in length, the relative lengths of the palpal

segments are 2 2, 5 7, 8 2, 10, 17 5, the pigmented area is mushroom-shaped, the buccal teeth number about 16, *a* is much greater than *γ*

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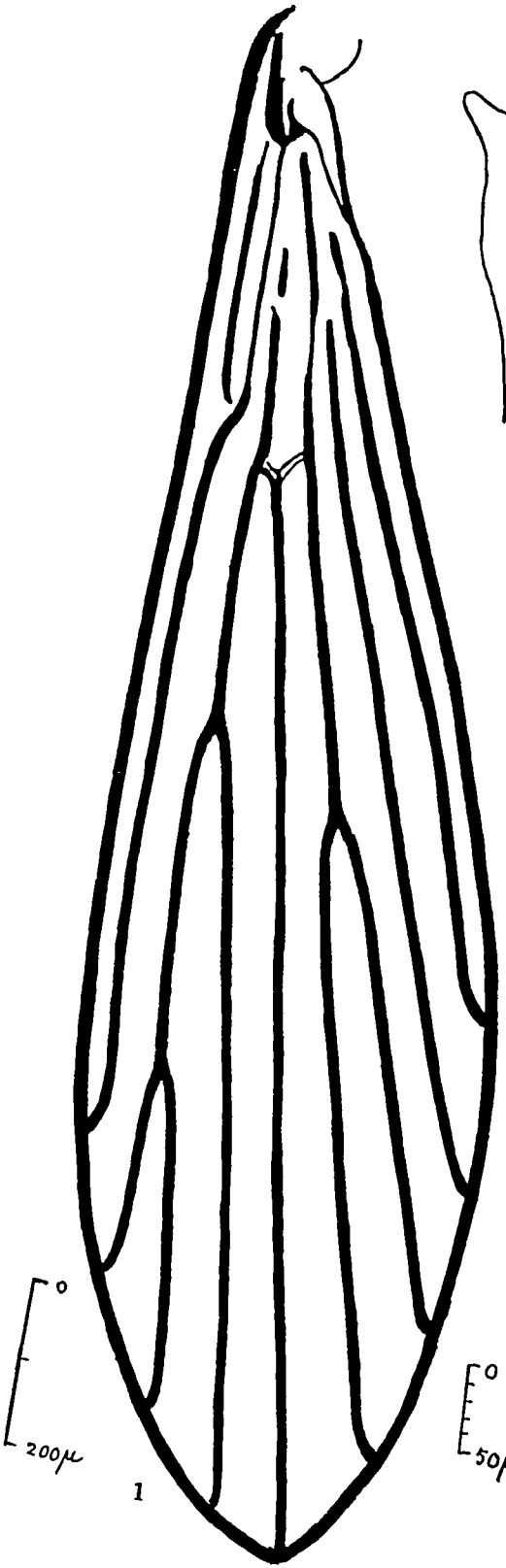
Explanation of Plate XXI

Phlebotomus eaditha (♀)

Fig 1 Wing
" 2 Buccal cavity
" 3 Post-genital plate
" 4 Antennal segments XII and XIII
" 5 Antennal segments III and IV
" 6 Palpal segments 1-4
" 7 Pharynx
" 8 Spermatheca



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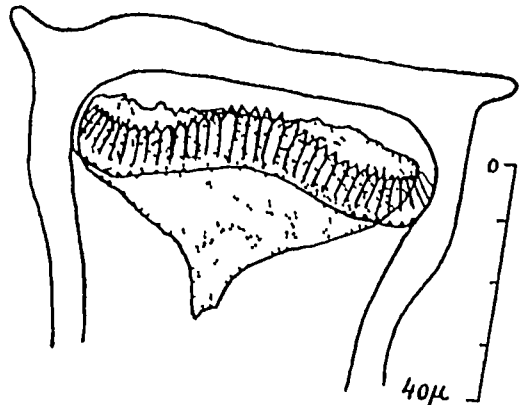

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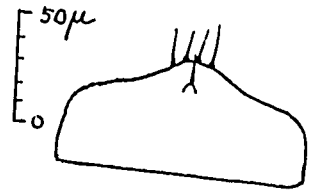

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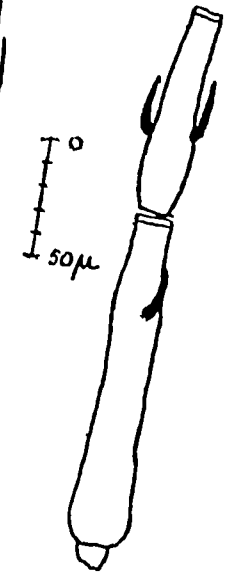

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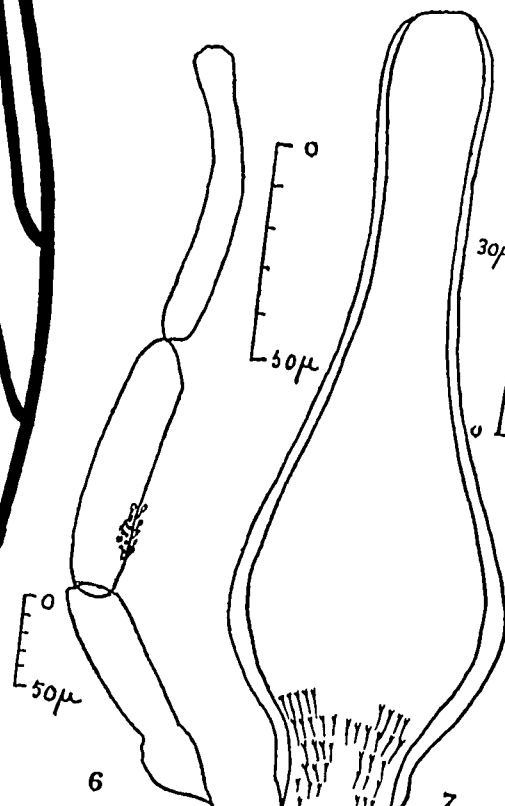

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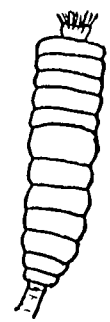

8

NOTE ON AN INTRADERMAL REACTION IN MONKEY MALARIA

BY

LIEUT-COLONEL J A SINTON, M D, D SC, I M S
Malaria Survey of India, Kasauli,

AND

CAPTAIN H W MULLIGAN, M D, I M S,
Central Research Institute, Kasauli

[Received for publication, August 10, 1932]

THROUGH the kindness of Lieut-Colonel R Knowles, I M S, Professor of Protozoology, and Lieut-Colonel H W Acton, C I E, I M S Director of the School of Tropical Medicine, Calcutta, we obtained a strain of monkey malaria from *Cercopithecus pygerythrus*, which had been found to be very virulent when inoculated into the common Indian monkey, *Macaca mulatta* (*Macacus rhesus*) Another strain of malaria has since been found by us in naturally infected monkeys and should form a useful control for specific experimentation

Various 'antigens' were prepared from the blood and organs of infected monkeys in an attempt to discover whether any serological reaction could be devised to show either infection with the parasite or evidence of changes in immunity produced by infection

During the course of these investigations, it was decided to try if any of the various 'antigens' prepared, would show any diagnostic changes, when injected intradermally into normal as compared with infected monkeys Very marked differences were obtained with some of these antigens

So far the most satisfactory results have been given by the use of an antigen prepared by digesting with papain a suspension of malarial parasites washed free of hæmoglobin The intradermal tests were made by injecting 0 05 to 0 10 c c of either the filtered digest or the redissolved deposit, which was obtained after this digest was added to a large excess of alcohol

In the following table are contrasted the results observed in normal and infected monkeys after intradermal injections of antigens of such strength that they caused little delayed reaction in normal monkeys

TABLE

DESCRIPTION OF INTRADERMAL REACTIONS IN NORMAL AND INFECTED MONKEYS

Time	Normal monkey	Infected monkey
Up to 3 minutes	Distinctly raised and very circumscribed, pale swelling, which quickly develops marked goose skin, often extending over surrounding area	Swelling much less distinctly raised and circumscribed Goose skin usually absent in acute infections, but may be slight in chronic cases, if present, it is seldom seen over the centre of the swelling Size of swelling much smaller than in normal control and it may be pink rather than whitish
15 minutes	Large circumscribed and raised swelling about 15–20 mm in diameter Goose skin marked, usually extending beyond swelling Seldom any redness and no petechiæ	Swelling smaller and less circumscribed than in normal Goose skin, if present, very scanty Redness of centre of swelling not uncommon Sometimes commencing petechiæ and cuticular necrosis
3–4 hours	Distinct fading of primary reaction Goose skin gone Swelling markedly subsided and area of primary reaction may have returned, more or less, to its original colour, or perhaps shows only a slight yellowish pallor More rarely the point of injection may have a pink, pin head papule	Marked increase in the degree and extent of reaction Pale yellowish or greenish pink, slightly raised button like area 10–25 mm in diameter with a smooth surface At the centre of this is a small area about 5 mm in diameter which may be deep pink and hyperæmic, purplish and petechial or even dark and necrotic, according to the severity of the reaction The periphery of the button like area has usually a distinct petechial areola of variable extent and intensity
24 hours	Usually only the mark of needle or a small pink papule left	Reaction is at its maximum, showing considerably greater intensity than at 3–4 hours Definite swelling about 20 mm diameter with central necrosis or petechiæ and destruction of superficial cuticle The petechial areola is usually wider and more intense, a condition which is a constant feature of this stage Usually there is distinct œdema of the surrounding tissues and the whole swelling is indurated
48 hours	Usually no signs	Usually little change since last observation, or perhaps commencing resolution, which may take a long time, especially if necrosis has been marked

It will be seen from a study of this table that the immediate reaction produced would appear to correspond to what Coca (1928) has classified as 'atopic hypersensitiveness', and this seems to be abolished to a greater or lesser degree by infection The delayed reaction seen in infected monkeys appears to be an example of 'hypersensitiveness of infection' (Coca, 1928) and to be very similar to the tuberculin reaction As in the latter reaction the severity seemed to have some relationship to the acuteness and severity of the infection, for it was less marked in monkeys suffering from chronic infections than in those with acute ones The amount of reaction in acute infections also seemed to be related to the number of parasites in the peripheral blood at the time the test was carried out

A total of 35 tests was made with various papain digests of blood and infected organs on 9 acutely and 4 chronically infected monkeys and 44 control tests were made on 9 normal monkeys There was a marked difference in all these injections between the results obtained in the diseased and the control animals

Further investigations will be necessary to determine whether this reaction is specific for one or all kinds of malaria in the monkey, how long it lasts after permanent cure, whether it has any bearing on the diagnosis of human malaria, etc

A fuller account of the work is being prepared for publication in the December number of the *Records of the Malaria Survey of India*

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Ind Jour Med Res, XX, 2, October, 1932

ALL-INDIA LIFE TABLES

BY

H P CHAUDHURI, M B (Cal U), D P H (Lond), D T M & H (Lond), F R S S (Lond)

(*Hony Research Worker, All-India Institute of Hygiene and Public Health, Calcutta*)

[Received for publication, June 13, 1932]

In this paper an attempt has been made to compile two sets of Life Tables, one for the Hindu and the other for the Mahommedan male population in India and to compare the mortality rates of these communities in a scientific manner The Life Table is an instrument which is as indispensable to the student of public health, as is the barometer or the thermometer to the student of meteorology That being so, its discussion cannot be out of place in a medical journal

Sir Athelstain Baines was responsible for two of the early Census Reports, but the credit of compiling the first Indian Life Table must be given to Sir George Hardy(1) whose investigations were based on the Census Returns of 1881, 1891 and 1901 Next came Auckland's(2) Table, the mortality experience obtained during the decennium 1901–1911, and this was followed by Meikle's(3) Mortality Table embodied in his report on the age distribution and mortality rates on the basis of the Census Reports of 1921 For the mortality rates of special classes of sections of the Indian population, reference may be made to the investigations of Catham(4), Thompson(5), Winter(6), Hunter(7) and Vaidyanathan(8)

We shall make no attempt to review all this literature, chiefly for the reason that most of the rates of mortality tables brought forward have been wholly lacking in generality They have been special tables, graduated to fit a particular set of observations often involving assumptions, which could not hold good in any general law of human mortality

In the construction of a Life Table two items of information are needed (1) the number exposed to risk of death, and (2) the deaths occurring in the number for each year of life The ratios of (2) to (1) give the yearly rate of mortality for the year in question

It is only in insurance practice that Life Tables are based on the experience of persons traced through life, otherwise a Life Table is usually constructed on —

(1) *Population*—the numbers of person of each sex at each age as ascertained by the Census Returns

(2) *Deaths*—the numbers of deaths at each year of age, registered for the year

(3) *Births*—quarterly or annual records of births

At infantile ages rates of mortality derived from Census Returns and Records of Deaths are unreliable To obtain a more accurate 'exposed to risk' at these ages, annual or quarterly records are necessary

Thus the $\frac{\text{deaths}}{\text{population}}$ gives the central death rate or m_x*

$$q_x = \frac{d_x}{p_1 + \frac{1}{2} d_1}$$

$$p_x = 1 - q_x, \quad l_x = p_x \times l_{x-1}$$

$$T_x = l_{x+1} + l_{x+2} + l_{x+3} \qquad \text{Whence } Oe_x = \frac{T_x}{l_x} + \frac{1}{2}$$

But the data obtained in India cannot be relied upon for the construction of a National Life Table, not only because the registration of births and deaths are defective or the ages recorded at death and census are inaccurate, but also because the Census Records are far from satisfactory There are also other difficulties in making an All-India National Life Table India is a vast country embracing diverse climates, communities and social conditions, and it is a country peculiarly subject to devastating epidemics and disastrous famines An experience, based upon the records of a few years or upon a selected place or community, may therefore be entirely misleading

It is to be noted that the figures in the tables compiled by Hardy, Auckland and Meikle were not actual figures, but census figures graduated mathematically Hardy's method of graduation was in accordance with Makeham's First Modification of Gompertz's Law of Human Mortality, into which he introduced a further modification of his own To express Makeham's First Modification algebraically $m_x = A + Bc^x$ where m_x represents the force of mortality at age x and A B, c are constants, the values of which are to be deduced from the facts, Hardy's own modification was $l_x = A + Hx + Bc^x + \frac{m}{nx+1}$ from which it follows —

$$L_x = \int_x^{x+1} l_x \, dx = \left(A + \frac{H}{2}\right) + Hx + \frac{Bc^x (c-1)}{\log_e c} + \frac{m}{n} \log_e \left(1 + \frac{n}{nx+1}\right)$$

Hardy used this for deducing the mortality rates from 0—12 He also deduced another graduation based on Makeham's First Modification of Gompertz's Law, of the 1901 figures by the special formula $\log N'_x = K + ax + bx^2 + mc^x$ where N'_x represented the 'population above age x' and $\log_{10} c$ was taken as $= 0\,039$ Makeham's Second Modification in the form of $\log T_x = K + ax + bx^2 + fx^3 + mc^x$, was used by Auckland Auckland's method of deducing the mortality rates from 30 to 90 consisted in applying this Second Modification with an abnormally high value of $\log_{10} c = 0\,058742$ ($c = 1\,1448$) calculated from the values of $\log_{10} l_x$ for graduating the l_x figures deduced by weighting the values of l_x in the Provincial figures according to the

* For explanation of symbols used in this paper *see* Appendix A

total male population of each Province (enumerated in 1911) The mortality rates from 13 to 30 were calculated by weighting the Provincial figures without graduation, deduced by $m_x = \frac{l_{x-1}-l_{x+1}}{2l_x}$ As for the mortality rates from 0—12, Auckland made use of Hardy's Modification

Meikle's method was based on Henry's suggestion, which consisted in summing the quinnary groups from the bottom upwards and comparing the resulting values with the corresponding values of T_x (Standard Table) and ratios calculated $\text{Log}\frac{T_x}{T_x \text{ (Standard Table)}}$ ratios curve is plotted and smoothed by $y=1-ax-bx^2+cx^3$, and this gives the age distribution of the population Thus, knowing the age distribution of two courses 1901 and 1921 and assuming the geometrical rate of increase, the population of the 6 months before and after the middle dates of the years of census record was calculated for each age, multiplying it by $\left(\gamma_x\right)^{\frac{1}{10}} \times 9\frac{1}{2}$ and $10\frac{1}{2}$ respectively Then by the formula $\frac{L_x}{L_{x+1}}=\frac{l_{x+\frac{1}{2}}}{l_{x-\frac{1}{2}}}=p_x-\frac{1}{}$ The values of q_x were obtained by $q_x = 1 - p_x$

Meikle made use of another method Assuming a constant rate of increase, $5Q_x=\frac{T_{x+5}-T_{x+10}}{T_x-T_{x+5}}$=probability of death within 5 years Then the ratios of $\frac{Q_x}{5\,Q_x}$ can be obtained from a standard table, and the same can be applied or q_x can be obtained graphically from 5 Q_x

The mortality rates deduced in the various ways given above from the Census Returns are but approximate estimates for normal conditions and thus they are not capable of satisfying the requirements of a Standard Life Table In the circumstances I had no alternative but to follow the method adopted by the Faculty of Actuaries The data(8*a*) I have used for preparing the appended tables consisted of figures representing —

(*a*) 'Lives under observation' of insurance companies in each year from 1905 to 1925 at each age from 19 to 95 (100,000)

(*b*) 'Years of risk', of which the number is the same as the number of lives exposed to risk An 'exposed to risk' for each year is obtained by taking the mean of the living at the beginning and at the end of the year, adding half the deaths and then combining them for the 20 years (848,744)

(*c*) 'Deaths' at each age of the lives exposed to risk within the period of observation, i e, deaths from the date of entry to the last date or to the previous death (13,440)

(*d*) 'Lives existent' at the close of observation and passing out of observation after their insurance had continued for a number of years (57,770)

Duration in the case of the 'died' can be obtained by taking the number of integral years between the date of purchase and the date of death Thus finding

the number of lives 'exposed to risk' and the number of 'deaths', we can calculate q_x without difficulty The ratios between the actual and the expected death rates, the latter being derived from a Standard Table, viz , the English Life Table for 1921, are to be applied, after being graduated by inspection, to the English Life Table, and the figures thus obtained are again to be graduated graphically This method is superior to the methods used by Hardy Auckland and Meikle, because, besides being simpler, it secures greater accuracy for the Life Tables by using actual figures, though these are limited only to the well-to-do and literate section of the population, the poor illiterate mass being, as a rule unable to take advantage of life insurance Thus, the appended Life Tables are not National Life Tables, but Life Tables of 'Select Class' In this connection it is to be noted that about 10 per cent of the male population is literate and that about 85 per cent of the assured lives are in normal health

The 'probability of living' rates for the Mahommedan population can be calculated from the Hindu figures by applying the ratios of $\frac{p_x \text{ (Mahommedans)}}{p_x \text{ (Hindus)}}$ at each age The p_x column being found the rest of the procedure becomes easy This particular p_x means the probability of living in the year 1921, which can be calculated from the p_x of each Provincial figure and weighting them according to the population From a careful study of the appended Life Tables we arrive at the following deductions —

(1) The Hindu mortality has decreased in the course of the last 20 years

(2) The Mahommedan mortality is lighter than the Hindus

(3) Addition of 9 years to English mortality of 1921 gives approximately the All-India Hindu mortality (from ages 20 to 95)

(4) In expectation of life an Indian life is 7 years shorter than that of an Englishman (from ages 20 to 95)

(5) One-fourth of the children die before the age of 1 year and half the population die within the age of 25 years

(6) Average expectation of life at birth is about 30

I am painfully conscious of the tersity of the above notes for which I crave the indulgence of my readers I should be guilty of ingratitude if I were not to express my obligations to Colonel Stewart for supplying me with necessary literature on the subject, and also for offering valuable suggestions and criticisms

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(2) On the estimated age distribution and rates of mortality (*Ibid* , **47**, p 315 *et seq*)

(3) Report on the age distribution and rates of mortality of 1921 census (*Government of India's Publication* 1926)

(4) On the mortality of Indian assured lives (*Trans Acturial Congress*, 1900, p 330 *et seq*)

(5) On the mortality of Indian assured lives (*T F A* , **1**)

(6) On the mortality of Indian assured lives (*Jour Inst Actuaries*, **43**)
(7) Experience of the lives assured with the New York Life Insurance Company from 1885—1892 (*Ibid*, **57**)
(8) Mortality of Indian assured lives (*Ibid*, **60**, p 180 *et seq*)
(8a) Experiences of Oriental Life Assurance Company mortality of Indian assured lives (*Ibid*, **60**, p 180 *et seq*)

In addition to the above papers the following were consulted for general guidance —

(9) 'Elderton's graduation of age distribution' (Elderton's book)
(10) Notes on the graduation of the age distribution tables by S J Cunningham, B Sc, F I A
(11) Notes on the graduation of the Punjab figures by S J Rowland, A I A
(12) Edgeworth's presidential address to the Royal Statistical Society (*Jour Royal Statis Soc*, 1913, pp 185–193)
(13) Mr G F Hardy's lectures on the construction and graduation of mortality tables (*Ibid*, pp 91–98)
(14) Athelstan Baine's paper on Census Report read before the Statistical Society
(15) Census Report of the year 1921
(16) Registrar General's decennial supplements of England and Wales, 1921, Part I, Life Tables

Appendix A

Explanation of Symbols

p_x = the probability of a person aged x living a year
q_x = the probability of a person aged x dying within a year
O_{ex} = the 'complete expectation of life', of a person aged x
m_x = central death rate
d_x = the deaths in the year of age x to $x + 1$ among the lx persons who enter in that year
l_x = the number of persons living between any two ages $x + x + 1$
T_x = the number of years lived by persons aged above x
$5Q_x$ = the probability of dying within 5 years
P_x = the population at age x to $x + 1$
L_x = the population at age x
γ_x = the rate of increase of the population at age x

Appendix B

As a further help to understand the structure and meaning of Life Tables, a nomogram, devised by Pearl and Reed, has been appended This nomogram has been constructed after the method described by these teachers in the Johns Hopkins School of Hygiene, Baltimore, U S A I have followed them descriptions and instructions in the method of construction and use

This is a diagram in two parts, plotted on a graph paper, one in which the abscissad divisons are arithmetically equal, and represent ages, and the ordinate divisons are proportional to the logarithms of the numbers set down by their side On the left hand, larger one of the two diagrams, there are plotted three lines, namely, dx, lx, and Tx, as defined above The right hand, or smaller one of the diagrams, is simply the same logarithmic scale as that of the larger of the two diagrams, but extending 1 to 1,000

How to use it?

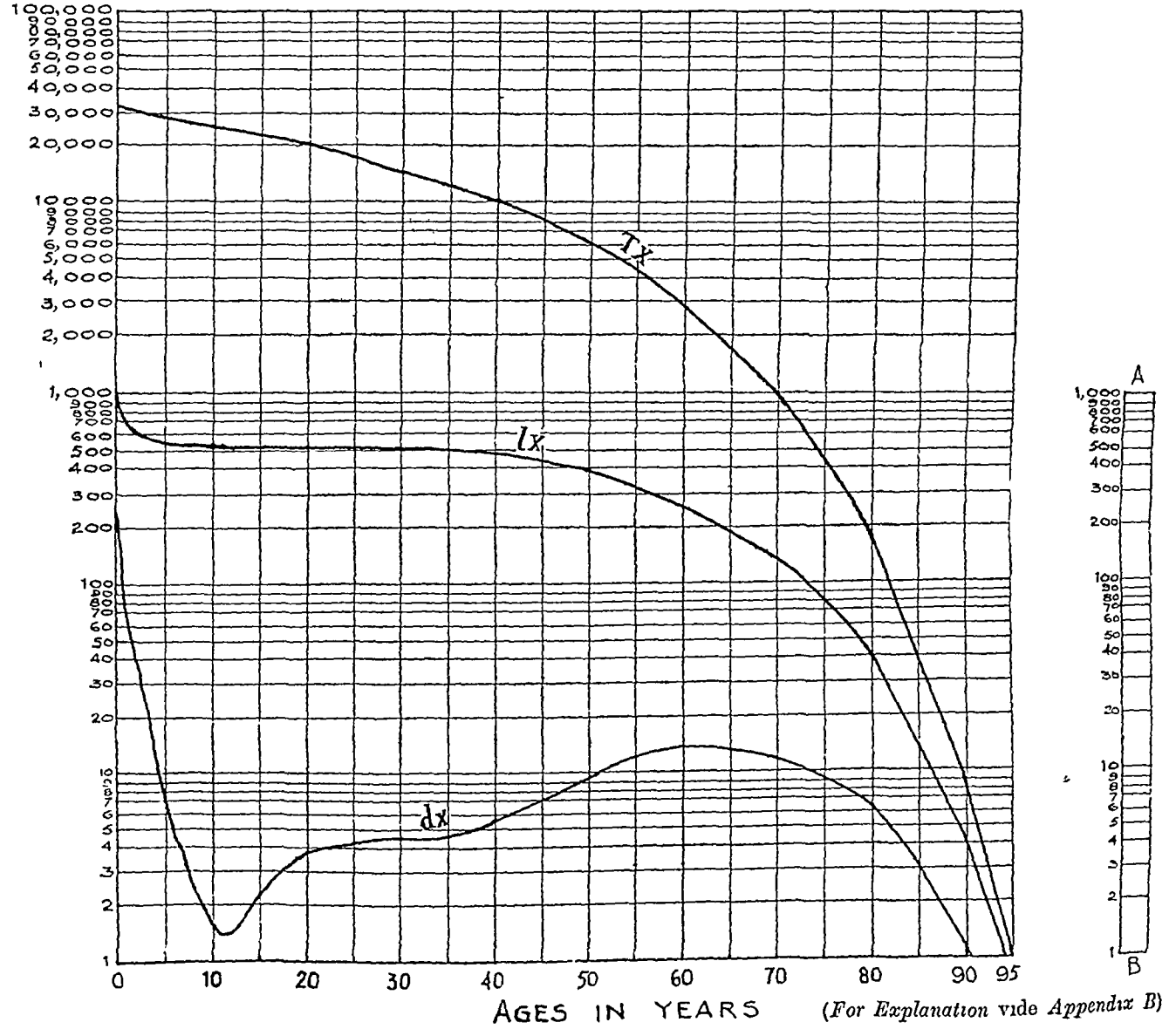
To find the death rate q_x place the scale on the nomogram in such a manner that A is on the l_x, line at the age x Then read the scale at the point, where at age x, it is cut by the d_x line of the nomogram The value so read will be q_x

To find the expectation of life O_{ex} place the scale in such a manner that B coincides with the l_x line, at age x Then read the scale at the point where, at age x, it is cut by the T_x line The value so read will be the expectation of life at age x

APPENDIX C

ALL-INDIA LIFE TABLE NOMOGRAM

GRAPH 1



(*For Explanation* vide *Appendix B*)

TABLE I

All-India Hindu Males Life Table

Age	p_x	l_x	T_x	O_{ex}	Age	p_x	l_x	T_x	O_{ex}
0	0 71625	100,000	3,118,135	30 58	49	0 97660	37,801	623,405	16 99
1	0 916809	71,625	3,018,135	42 62	50	0 97487	36,916	595,604	16 63
2	0 942540	65,667	2 946,510	45 37	51	0 97291	36 851	568,688	15 93
3	0 96010	61,894	2,880 843	47 04	52	0 97077	35 853	532,835	15 36
4	0 972515	59 424	2 818 949	47 93	53	0 96842	34,805	498,030	14 80
5	0 981107	57,791	2,759 525	48 25	54	0 96595	33,706	464,324	14 27
6	0 98696	56,699	2,702,826	48 16	55	0 96337	32 558	431 766	13 76
7	0 99100	55 960	2,646,866	47 79	56	0 96067	31,365	400,401	13 28
8	0 99353	55,456	2 591,410	47 22	57	0 95791	30,131	370,270	12 78
9	0 99521	55 098	2,536,312	46 53	58	0 95505	28,863	341,407	12 32
10	0 99730	54,834	2,481,478	45 75	59	0 95191	27,566	313,841	11 85
11	0 99780	54,686	2,426,792	44 87	60	0 94903	26,240	287,601	11 46
12	0 99790	54,566	2 372,226	43 97	61	0 94612	24,903	262 698	11 16
13	0 99760	54,451	2 317,775	43 16	62	0 94312	23,561	239,137	10 64
14	0 99720	54,320	2,263,455	42 16	63	0 93994	22,221	216,916	10 36
15	0 99660	54,168	2,209,287	41 28	64	0 93659	20,886	196,030	9 89
16	0 99580	53,939	2,155,348	40 45	65	0 93315	19,562	176,468	9 52
17	0 99510	53,712	2,101,636	39 62	66	0 92949	18,254	158,214	9 16
18	0 99425	53,449	2,048,187	38 81	67	0 92567	16,967	141,247	8 82
19	0 99350	53,141	1,995,046	38 04	68	0 92169	15,706	125,541	8 48
20	0 99275	52,796	1,942,250	37 28	69	0 91765	14,476	111,065	8 14
21	0 99259	52,413	1,889,937	36 55	70	0 91357	13,284	97,781	7 86
22	0 99240	52,025	1,837,812	35 82	71	0 90919	12,136	85 645	7 55
23	0 99219	51,630	1,786,182	35 09	72	0 90440	11,033	74 612	7 17
24	0 99200	51,227	1,734,955	34 37	73	0 89907	9,978	64 631	6 97
25	0 99183	50,817	1,684,138	33 64	74	0 89335	8,971	55 663	6 70
26	0 99168	50,402	1,633,736	32 91	75	0 88746	8,013	47,650	6 45
27	0 99154	50,100	1,583,636	32 10	76	0 88068	7,111	40,539	6 20
28	0 99140	49,676	1,533,960	31 37	77	0 87356	6,263	34,276	5 97
29	0 99127	49,249	1,484,711	30 64	78	0 86568	5,471	28,013	5 62
30	0 99113	48,819	1,435,892	29 91	79	0 85739	4,736	22,542	5 25
31	0 99097	48 378	1,387,514	29 18	80	0 84855	4,061	17,806	4 88
32	0 99080	47,941	1,339,573	28 44	81	0 83924	3,446	13,745	4 50
33	0 99058	47,500	1,292,073	27 70	82	0 82937	2,892	10,853	4 25
34	0 99032	47,039	1,245,034	26 96	83	0 81865	2,399	8,454	4 02
35	0 98999	46,584	1,198,450	26 22	84	0 80839	1,964	6,490	3 81
36	0 98960	46,117	1,152,333	25 48	85	0 79649	1,588	4,902	3 58
37	0 98915	45,637	1,106,696	24 95	86	0 78470	1,266	3,636	3 37
38	0 98861	45,142	1,061,554	24 01	87	0 77040	993	2,643	3 16
39	0 98804	44,628	1,016,926	23 26	88	0 75875	765	1,878	2 95
40	0 98737	44,064	972,862	22 58	89	0 74264	580	1,298	2 73
41	0 98661	43,507	929,355	21 86	90	0 73110	424	874	2 56
42	0 98575	42,924	886,431	21 15	91	0 72350	310	564	2 31
43	0 98480	42,312	844,119	20 45	92	0 70827	217	347	2 09
44	0 98374	41,624	802 495	19 77	93	0 68857	149	198	1 82
45	0 98257	40,898	761,597	19 12	94	0 63733	102	96	1 44
46	0 98126	40,186	721,411	18 40	95	0 45700	65	31	0 97
47	0 97985	39,433	681 978	17 79	96	0 05003	29	2	
48	0 97834	38,638	653,340	17 56	97		2		

Table II

All-India Mahommedan Males Life Table

Age	p_x	l_x	T_x	O_{ex}	Age	p_x	l_x	T_x	O_{ex}
0	0 71124	100,000	2,947,930	29 97	49	0 98016	36,005	626,472	17 89
1	0 91180	71,124	2,876,806	40 94	50	0 97848	35,290	591,182	17 25
2	0 93753	64,851	2,811,955	43 86	51	0 97657	34 531	556,651	16 60
3	0 95509	60,800	2,751 155	45 74	52	0 97436	33 722	522,929	16 00
4	0 96750	58,124	2,693,031	46 83	53	0 97185	32,857	490 072	15 41
5	0 97600	56,235	2,634,907	47 35	54	0 96874	31,932	458,140	14 84
6	0 981000	54,885	2,580 022	47 53	55	0 96597	30,934	427,206	14 31
7	0 98504	53,842	2,526 180	47 41	56	0 96320	29,881	397,325	13 79
8	0 98757	53,036	2,473,144	47 13	57	0 96045	28,781	368,544	13 30
9	0 98925	52,377	2,420,767	46 72	58	0 951965	27,643	340,901	12 83
10	0 99371	51,814	2,368,953	45 72	59	0 95400	26,472	314,429	12 37
11	0 99421	51,488	2,317 465	45 50	60	0 95254	25,254	289,175	11 95
12	0 99431	51,189	2,266 276	44 77	61	0 95204	24,056	265,119	11 70
13	0 99423	50,898	2,215,378	44 02	62	0 94929	22,902	242,217	11 07
14	0 99415	50,604	2,164,774	43 27	63	0 94629	21,740	220 477	10 64
15	0 99407	50,308	2,114,466	42 53	64	0 94300	20,572	199,905	10 17
16	0 99399	50,010	2,064,466	41 78	65	0 93763	19 399	180,506	9 80
17	0 99372	49,709	2,014,737	41 03	66	0 93463	18,189	162 317	9 42
18	0 99326	49,397	1,965,360	40 28	67	0 93127	17,000	145,317	9 04
19	0 99261	49,064	1,916,296	39 55	68	0 92755	15,831	129 486	8 67
20	0 99176	48,701	1,867,595	38 84	69	0 92347	14,684	114,802	8 31
21	0 99175	48,300	1,819,295	38 16	70	0 91905	13,561	101,211	7 96
22	0 99174	47,902	1,771,393	37 47	71	0 91445	12,463	88,778	7 62
23	0 99173	47,506	1,723,887	36 78	72	0 90955	11,397	77,381	7 28
24	0 99172	47,113	1,676,774	36 09	73	0 90435	10,366	67,015	6 96
25	0 99171	46,723	1,630,051	35 38	74	0 89885	9,374	57,641	6 64
26	0 99170	46,334	1,583,717	34 68	75	0 89304	8,426	49,215	6 34
27	0 99169	45,949	1,537,768	33 96	76	0 88774	7,532	41,683	6 03
28	0 99167	45,567	1,492,201	33 24	77	0 88094	6,687	34,996	5 74
29	0 99165	45,187	1,447,014	32 52	78	0 87334	5,890	29,106	5 44
30	0 99163	44,810	1,402,204	31 79	79	0 86494	5,144	23,962	5 15
31	0 99150	44,434	1,357,770	31 05	80	0 85593	4,449	19,513	4 88
32	0 99153	44,060	1,313,710	30 31	81	0 84663	3,808	15,705	4 74
33	0 99142	43,686	1,270,024	29 57	82	0 83693	3,201	12,504	4 40
34	0 99128	43,310	1 226,714	28 82	83	0 82673	2,679	9,825	4 16
35	0 99108	42,932	1,183,782	27 97	84	0 81590	2,214	7,611	3 93
36	0 99084	42,549	1,141,233	27 32	85	0 80461	1,807	5,804	3 71
37	0 99054	42,159	1,099,074	26 57	86	0 79301	1,454	4,350	3 49
38	0 99018	41,760	1,057,314	25 81	87	0 78081	1,153	3,197	3 27
39	0 98976	41,350	1,015,964	25 12	88	0 76791	900	2,297	3 05
40	0 98915	40,926	975,038	24 32	89	0 75441	691	1,606	2 82
41	0 98857	40,482	934,112	23 58	90	0 73973	521	1,085	2 58
42	0 98789	40,019	893,630	22 83	91	0 72470	385	700	2 31
43	0 98701	39,535	853,611	22 09	92	0 69470	279	421	2 00
44	0 98603	39,021	811,076	21 36	93	0 63939	194	227	1 67
45	0 98522	38 476	775,609	20 65	94	0 55939	124	103	1 33
46	0 98422	37,907	737,693	19 96	95	0 46319	69	34	0 99
47	0 98304	37,309	699,786	19 25	96	0 05023	32	2	
48	0 98169	36,676	662,477	18 56					

Table III

Comparison of mortality

(a) By probability of surviving ten years, $10\ p_x$

Age	England and Wales 1921	All India Hindu Males 1925	All India Mahommedan Males 1925	All India 1911	All India 1901
0	0 85693	0 5483	0 1572	0 5021	0 5103
10	0 97730	0 9028	0 9399	0 8729	0 8849
20	0 96180	0 9246	0 9201	0 8174	0 8447
30	0 94718	0 9025	0 9133	0 7573	0 7802
40	0 91640	0 8578	0 8622	0 6875	0 6946
50	0 84117	0 6941	0 7156	0 6055	0 5946
60	0 67217	0 5062	0 5369	0 4543	0 4288
70	0 38038	0 3057	0 3280	0 2010	0 1727
80	0 11373	0 1044	0 1171	0 1065	0 1637

(b) By the number of survivors, l_x

Age	England and Wales 1921	All India Hindu Males 1925	All India Mahommedan Males 1925	All India 1911	All India 1901
0	100 000	100,000	100 000	100,000	100 000
10	85,693	54,834	51,814	50 212	51,034
20	83,748	52,796	47,701	43 833	45,161
30	80,549	48,819	44,810	35 831	38 151
40	76,294	44 064	40 926	27,136	29,766
50	69 916	36,915	35 290	20,678	18,658
60	58 804	26,240	25,254	12 297	11 229
70	39 526	13,284	13,561	5 273	5,134
80	15,035	4,061	4,449	916	236
90	1,710	424	1,085	15	11

(c) By the death rates, Q_x

Age	England and Wales 1921	All India Hindu Males 1925	All India Mahommedan Males 1925	All India 1911	All India 1901
0	0 08996	0 28375	0 28876	0 2900	0 2854
10	0 00181	0 00270	0 00629	0 0125	0 0124
20	0 00349	0 00725	0 00824	0 0169	0 0143
30	0 00434	0 00887	0 00837	0 0235	0 0202
40	0 00688	0 01263	0 01085	0 0323	0 0301
50	0 001179	0 02513	0 02152	0 0428	0 0430
60	0 02561	0 05097	0 04716	0 0598	0 0625
70	0 05997	0 08643	0 08095	0 1037	0 1126
80	0 14002	0 15145	0 14407	0 2287	0 2355
90	0 26752	0 26890	0 26027	0 5455	0 4786

(d) By expectation of life, O_{ex} (years)

Age	England and Wales 1921	All India Hindu Males 1925	All India Mahommedan Males 1925	All India 1911	All India 1901
0	55 62	30 68	29 97	22 59	23 63
10	54 64	45 75	45 72	33 36	34 73
20	45 78	37 28	38 84	27 46	28 59
30	37 40	29 91	31 79	22 10	22 90
40	29 19	22 58	24 32	18 02	17 91
50	21 36	16 65	17 25	13 97	13 59
60	14 36	11 46	11 95	10 00	9 53
70	8 75	7 86	7 96	6 17	5 80
80	4 93	4 88	4 88	3 04	3 07
90	2 82	2 36	2 58	1 23	1 23

TABLE IV

Comparison of the Life Table I, with that of England and Wales, 1921

All India Hindu Males Age	Corresponding England and Wales Age	Difference in in age Older (+)	Expectation of life in years	Ratio of mortality of $\frac{q_x}{q_{x+9}}$ in 1921
0	25	+ 25	30 50	
5	15	+ 10	49 0	
10	20	+ 10	46 0	
15	25	+ 10	41 0	
20	29	+ 9	37 0	+ 1 73
25	34	+ 9	33 5	+ 1 54
30	21	+ 9	30 0	+ 1 35
35	43	+ 8	26 0	+ 1 19
40	48	+ 8	22 5	+ 1 15
45	53	+ 8	19 0	+ 1 07
50	57	+ 7	16 5	+ 1 05
55	61	+ 6	14 0	+ 1 00
60	65	+ 5	11 5	+ 0 930
65	69	+ 4	9 5	+ 0 775
70	72	+ 2	8 0	+ 0 667
75	76	+ 1	6 5	+ 0 598
80	80	0	5 0	+ 0 599
85	86	+ 1	3 5	+ 0 608
90	92	+ 2	2 5	+ 0 620
95	100	+ 5	1 00	+ 0 930
		Mean + 7		Mean + 1 00

Average—Rate of mortality is same as that of England and Wales in 1921 with 9 years added to the age from 20 to 90 In expectation of life + 7 years older

TABLE V

MALES

Comparison of probability of living of Mahommedans with that of Hindus (M-H), by ratio of p_x to p_x (M-H)

Ages	Bengal	Punjab	Madras	Bombay	U P	Graduated All India
0						0 9930
5	0 9933	0 9950	0 9991	0 9993	0 9996	0 9948
10	0 9972	0 9970	0 9978	0 9996	0 9981	0 9964
15	0 9954	0 9972	0 9957	0 9990	0 9999	0 9978
20	0 9970	1 0002	0 9955	0 9988	1 000	0 9990
25	0 9983	1 0026	0 9960	0 9988	0 9999	1000
30	0 9997	1 0025	0 9978	0 9954	0 9999	1 0005
35	1 0013	1 0018	0 9974	0 9994	1 0025	1 0071
40	1 0023	1 0018	0 9968	0 9992	1 0037	1 0018
45	1 0043	1 0022	0 9980	0 9998	1 0076	1 0027
50	1 0048	1 0062	0 9990	1 0003	1 0070	1 0037
55	1 0045	1 0050	0 9993	1 0022	1 0093	1 0048
60	1 0050	1 0116	1 0012	1 0038	1 0116	1 0060
65	1 0055	1 0104	1 0042	1 0039	1 0126	1 0073
70						1 0087
75						1 0112
80						1 0118
85						1 0135
90						1 0153
95						1 0172

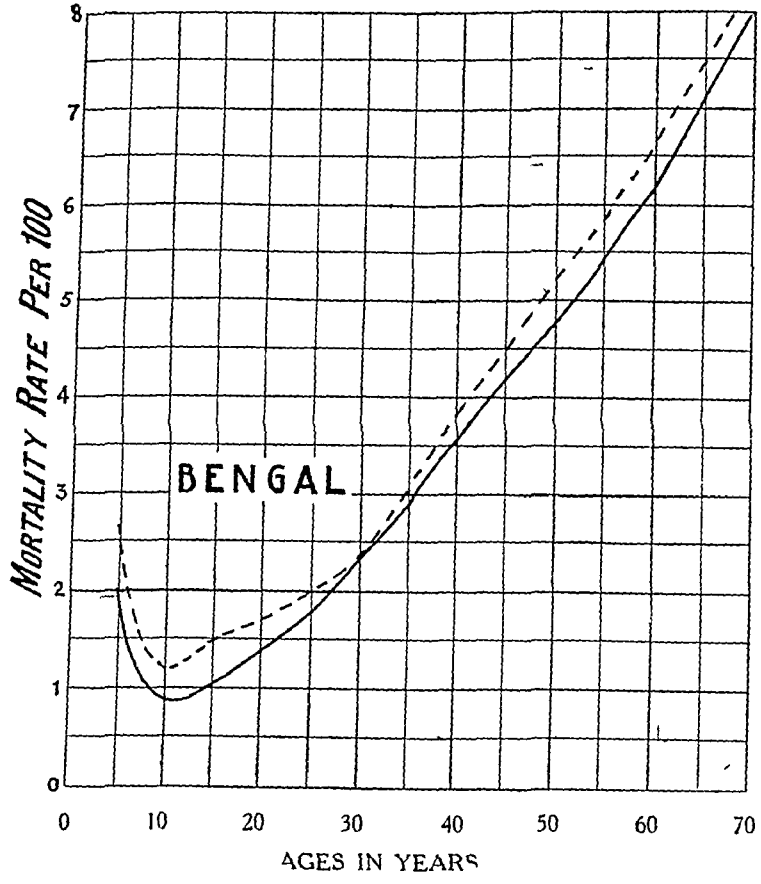
Mahommedan mortality compared with Hindu mortality shows --

HEAVIER (1) Up to age 30, Bengal (2) Up to age 15 Punjab, Madras

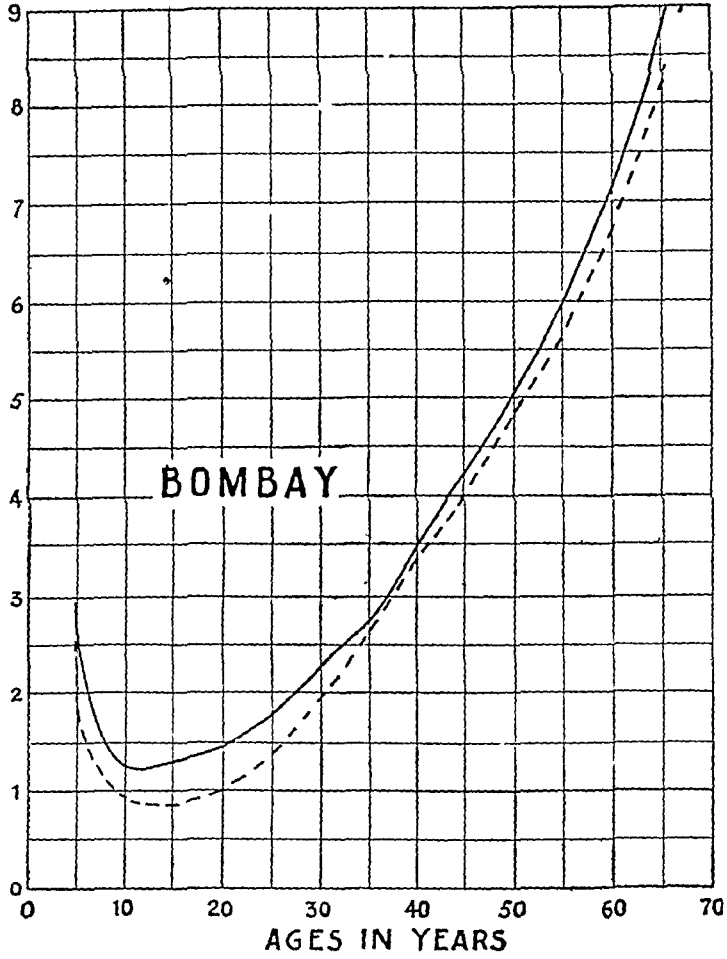
LIGHTER (1) At all ages Bombay, United Provinces (2) From 30 upwards Bengal (3) From 15 upwards Punjab, Madras It has also been shown graphically

SPECIFIC DEATH RATES, 1921

GRAPH 2



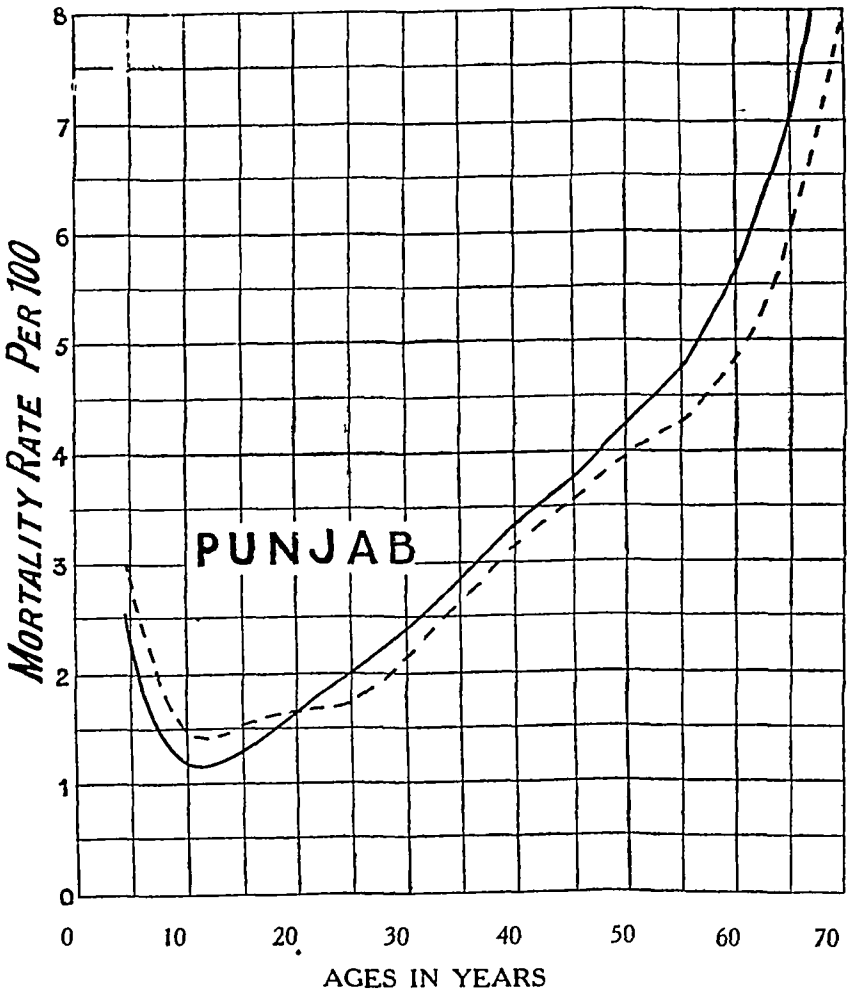
GRAPH 3



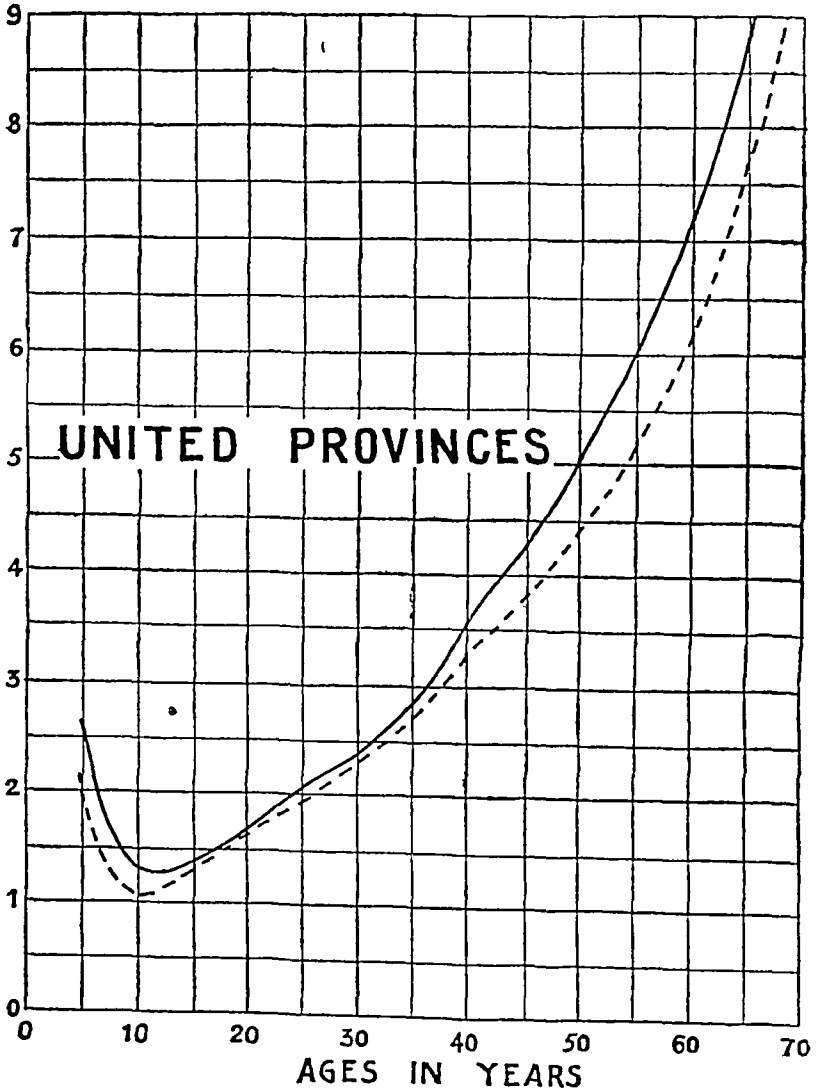
Unbroken Lines = Hindus Broken Lines = Mahommedans

SPECIFIC DEATH RATES, 1921.

GRAPH 4

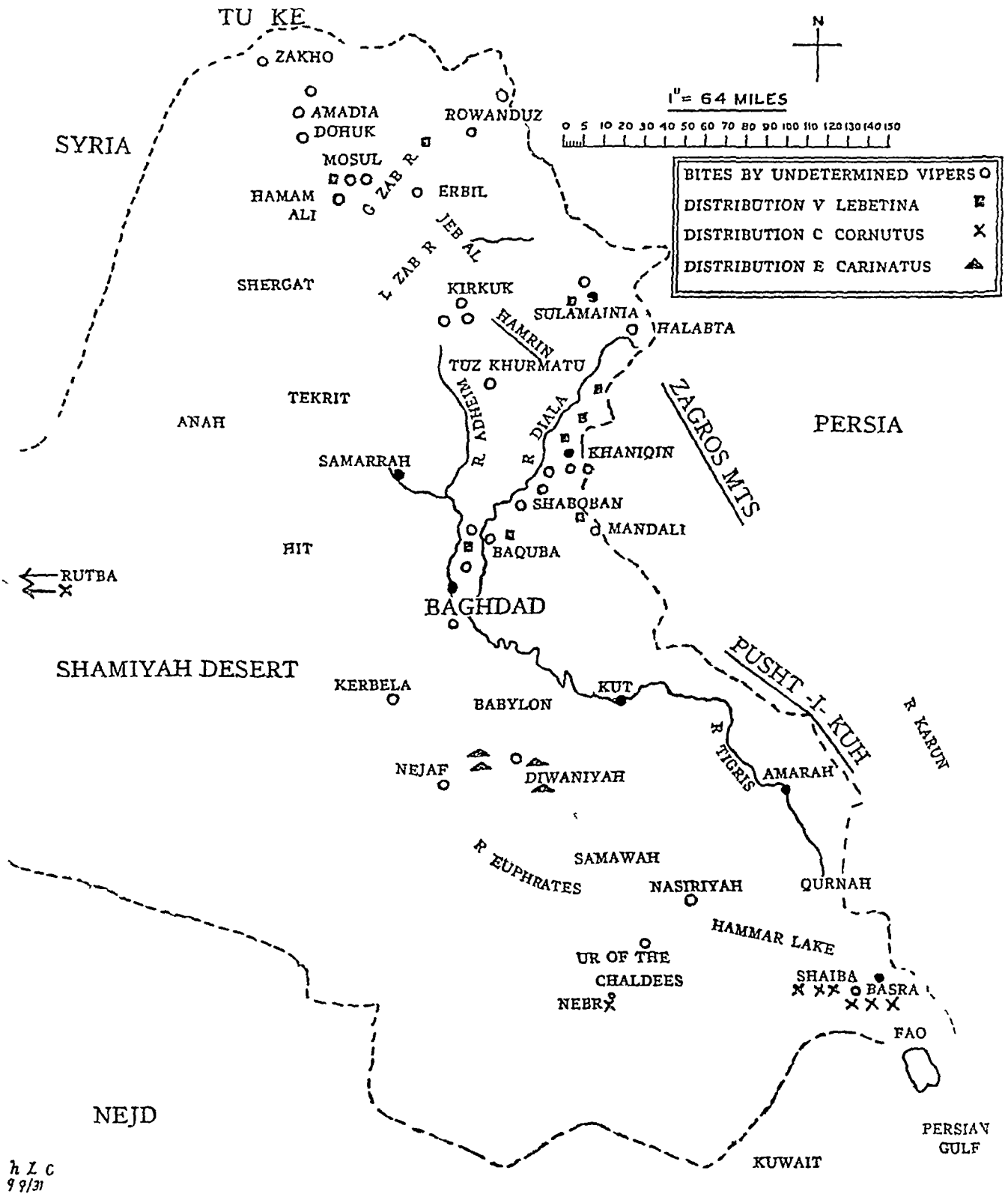


GRAPH 5



Unbroken Lines = Hindus Broken Lines = Mahommedans

MAP



Sketch Map of Iraq to show the distribution of viperine species and also the distribution of cases of viperine poisoning from bites by undetermined snakes

Ind Jour Med Res, XX, 2, October, 1932

AN INQUIRY INTO SNAKE-BITE IN IRAQ

BY

NORMAN L CORKILL, M B , Ch B , T / S ,
Sudan Medical Service
(Late Iraq Health Service)

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Introductory remarks

Bites by venomous snakes in Iraq do not constitute the ever present menace to life and mental ease that they do for example in parts of India Nevertheless cases occur from time to time and the dramatic symptoms (for most of such cases are viperine), and only too frequent fatal termination, create a wish on the part of any medical officers concerned to know what poisonous snakes are to be found in their areas, how they may readily be identified, what characteristic symptoms result from their bites, what treatment has been found of value and what the prognosis may be The appreciation of such a desire was responsible for the amassing of the information contained in this paper

The inquiry has two aspects Firstly the zoological, it being an obvious prime necessity to establish the poisonous species occurring in Iraq, having particular regard to their distribution , it is barely less necessary to ascertain also the species and distribution of the non-venomous snakes so that an easy key to field identification may be formulated Incidentally this aspect has comprehended a study and collection of the vernacular names of snakes In most cases of snake-bite, the offender is not secured , it may, however, have been seen and if its name as mentioned by an onlooker of the accident is attachable to a definite species, useful information has been obtained

An examination of existing records and material is obviously the first desideratum It may then be supplemented

The second aspect of the inquiry is a medical one It entails an examination of the literature on the venoms of, and cases of snake-bite by, the poisonous species, and its supplementing by the collection of records of bites from several sources, namely, the British Army medical records for the Great War, the Royal Air Force Medical Service, the Medical Department, Ministry of Defence, Iraq, and, as might

be expected, the most fruitful source of all, the records of the Iraq Health Service A certain number of cases were collected from unofficial sources but were usually not accepted as evidence A certain amount of quaint folklore relating to snakes and snake-bite was revealed in the course of these inquiries It is hoped to publish this elsewhere in a separate paper

The purely zoological data that resulted from the examination of over a hundred or so specimens and that have no relevance here, have been sent to the Bombay Natural History Society The inquiry extended from the autumn of 1927 to the early spring of 1930

A total of twenty-four species of snakes was established for Iraq They were two members of the family Typhlopidæ, one of the Leptotyphlopidæ, one of the Boidæ, eleven of the aglyphous colubrides, four of the opisthoglyphous colubrides, one of the Hydrophiidæ (old Proteroglypha, Hydrophiinæ), one of the Elapidiæ (old Proteroglypha, Elapinæ), and three of the Viperidæ The opisthoglyphous snakes are not considered here as poisonous, though it is frankly admitted that their right to omission is debatable However, there is no evidence at present to be offered one way or the other as to whether such species in Iraq may be considered dangerous

Five species are considered dangerously venomous They are the Hook-nosed Sea-Snake, *Enhydrina schistosa*, the Hoodless Cobra, *Naja morgani*, the Levantine or Blunt-nosed Viper, *Vipera lebetina*, the Horned Viper, *Cerastes cornutus*, and the Saw-scaled Viper, *Echis carinatus*

It is pertinent to summarize the physical characters of Iraq The present political kingdom, which is the area embraced by this inquiry, includes classical Mesopotamia or 'land between the two rivers' the neighbouring plains, and also the broken ground, hills and mountains, north and east of Baghdad, that is, the Jebal Hamrin and the heights of Kurdistan ascending to Anatolia and the Persian Plateau The silt plain from Hit and Samarra stretches away to the Persian Gulf in the South, threaded by the ancient rivers, the Tigris and the Euphrates The lower course of the latter is beset with lake, marsh and varying channels Before the rivers reach Basra in the south they join Their waters flow on as the Shatt-el-Arab (River of the Arabs), are joined below Basra by the Karun River of Persia and empty into the Persian Gulf at Fao An adjoining strip of territory gives Iraq its coast West of the Euphrates the Shamiyah and Southern Deserts roll away in a gradual rise to Nejd On the east the ground from the Tigris ascends to the Zagros Mountains and the Persian Pusht-i-Kuh

The plains suffer in summer such degrees of shade temperature as 128°F, in the winter frozen puddles may be seen on the roads Rain is limited to about ten or so wet days in the winter In Kurdistan and the north-east more coolness and rain are the natural accompaniments of altitude, more northerly latitude and increased vegetation

Four types of habitat will be obvious the marine waters of the Persian Gulf and Shatt-el-Arab estuary, the riverain cultivation and marsh, the desert plains, and the broken ground ascending to Anatolia and Persia in the north-east

The population of Iraq is less than three millions and is massed mostly in the towns and villages along the rivers It is probable that this portion of the people is not more subjected to snake-bite accidents than the portion which is nomadic, or inhabits the less accessible places where medical aid is none too easily procurable It is therefore probable that any statistics of snake-bite cases obtained will provide an underestimate of the danger to man from snake-bite in Iraq

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THE POISONOUS SNAKES OF IRAQ

There is little literature on the snakes of Iraq as such, though that of certain of the individual species is very extensive However, the Bombay Natural History Society issued a small pamphlet in 1916 for the benefit of the interested members of ' Expeditionary Force D ' and a certain amount of information on snakes already recorded from Iraq or likely to be found there was included There was a most commendable response to this pamphlet and the material amassed during the War was published in a series of papers in the Journal of the Society These papers were ultimately collected into one volume—' A Survey of the Fauna of Iraq ' Two of the papers dealt with snakes The first one by Boulenger (1920) contained records for the first time from Iraq of *Naja morgani*, *Vipera lebetina* and *Cerastes cornutus* The second paper by Miss Procter (1921) contained no reference to poisonous species I know of no literature referring to records of sea-snakes taken in the neighbourhood

* *See* January 1933 number of the *Journal* (In the Press)

of the Shatt-el-Arab estuary, but Blanford (1876) and Malcolm Smith (1926) describe species that occur in the Persian Gulf

Sinderson (1924) recorded the occurrence of *Echis carinatus* in the Middle Euphrates area

Family HYDROPHIIDÆ

The Hook-nosed Sea-Snake, **Enhydrina schistosa** (Daudin)

The species was first described as *Hydrophis schistosus* by Daudin (1803) as an inhabitant of the Indian seas In 1876 Blanford referring to it under the synonym *Enhydrina valakadyen* Boie , recorded its occurrence at Gwadar on the Baluchistan coast Boulenger recorded in 1896 a specimen from Muscat under the synonym *Enhydrina valakadien* In 1926 Malcolm Smith published records from Karachi, Persian Gulf and Muscat

In 1929 four black-banded, bluish-grey sea-snakes were discovered preserved in spirit in the Museum room of the Agricultural College at Rustum Farm, south of Baghdad They were labelled '*Hydrophis cyanocincta*—Persian Gulf, 1921' and were assumed to be duplicates of the Cox-Cheesman collection made about that time

Hydrophis cyanocinctus according to Malcolm Smith (1926) is characterized by 37–47 rows of scales at mid-body which are imbricate throughout and usually have a central keel which 'may be broken into a series of two or three tubercles' The same writer characterizes *Enhydrina schistosa* by 49–66 scales at mid-body, the scales imbricate or sub-imbricate and with a short central keel Both snakes may be pale below and with a bluish-grey dorsum banded black, as was the colour of the four specimens found at Rustum Farm

These tallied perfectly with each other as regards appearance and proportions The scales, which were sub-imbricate, bore the very faintest suggestion of central keeling and numbered 54 rows at mid-body in the only specimen 'counted' The rostral turned down under the upper lip and projected over the lower jaw This is presumably the characteristic giving the reptile its popular name 'The Hook-nosed Sea-Snake' (*see* Plate XXII, facing page 613) I have felt justified in recording the species as inhabiting the marine waters of Iraq It is possible that it may appear in the Shatt-el-Arab at Basra, as Finn (1929) has recorded its occurrence in an Indian river eighty miles upstream from the coast

According to Malcolm Smith it is the commonest sea-snake known and is distributed from the Persian Gulf to the coast of Cochin-China and the north coast of Australia I know of no vernacular names that would apply to it in Iraq other than the 'Haiat-al-Bahr', that is literally the 'snake of the sea'

An engineer from one of the dredgers employed at the bar of the Shatt-el-Arab off Fao told me that it was no uncommon thing for sea-snakes about four feet long to be emptied out of the sludge buckets with their other contents He said that

these snakes were invariably greenish-grey with black bands They were known to the native crew as being poisonous

It may be of passing interest to note that other species of Hydrophidæ occurring in the Persian Gulf are —

Thallasophina viperina (Schmidt) Persian Gulf and Muscat
Hydrophis spiralis (Shaw) Persian Gulf, Gangestum and Muscat
Hydrophis cyanocinctus (Daudin) Persian Gulf
Hydrophis ornatus (Gray) Muscat
Hydrophis lapemoides (Gray) Persian Gulf and Jask
Lapemis curtus (Shaw) Muscat
Microcephalophis gracilis (Shaw) Persian Gulf and Gulf of Oman

With the exception of the last named all might give the impression of being greenish-grey, banded black However there are no definite records of their association with the Gulf as far north as Fao

Family ELAPIDÆ

The Hoodless Cobra, **Naja morgani** Mocquard

Mocquard (1905) described *Naja morgani* as a new species from five specimens brought from 'Perse (Arabistan)' by M Morgan Wall (1908) described a black snake from Persia (Maidan Mihaften, I believe) as *Atractaspis wilsoni* This name was given as a synonym of *N morgani* by Boulenger (1920) when recording the occurrence of the latter in Iraq, on specimens from 'Shaiba and Mesopotamia' collected by members of Expeditionary Force 'D'

When talking to Mr W H Parker, Assistant Keeper (Amphibians and Reptiles), at the British Museum in 1930 my attention was drawn to the rarity *Walterinnesia ægyptia* Lataste, in view of my forthcoming departure for the Sudan I was shown it figured in Anderson's classic and immediately remarking on its superficial resemblance to *N morgani*, determined to follow the matter further The first thing to do was to establish a prima facie case for relationship, thereafter to examine the generic status of both species and then proceed to a detailed examination and comparison of all available specimens of both The exigencies of time and circumstance have permitted little other than the formulation of a good prima facie case Mr W H Parker was good enough to examine the British Museum specimens of *W ægyptia* and *N morgani* for the presence of the small teeth behind the maxillary fangs, upon the existence of which generic validity depends *Naja morgani* was proved to be undoubtedly a Naja The specimen of *W ægyptia* examined certainly showed no teeth behind the fang on the side dissected, but there was a roughened area present on the maxilla suggesting that it is not impossible that teeth were there originally Pending further investigation I considered a Scots verdict admissible and still think a detailed comparison of interest

Lataste (1887) described the species *Walterinnesia ægyptia* from, I believe, two specimens They were purchased from a snake-charmer in Cairo by Dr Walter Innes, who stated that the vendor had declared their Arabic name to be 'Bargil' One of these specimens is in the British Museum and is, I presume, the specimen on which the descriptions of Boulenger (1896) and Anderson (1898) are based Flower (1923) first described a specimen with a satisfactorily established provenance, the Cairo-Suez road Mertens (1929) writing of a further specimen in the Senckenberg Museum said it was one of six specimens known and the second one only, of which the place of collection had been precisely stated, the first one being that of Flower's description above , the provenance was the same, the Cairo-Suez road

In 1929 Tewfiq Begal Naqib of Baghdad and Basra was good enough to compile for me a list of the Arabic names of snakes used in Iraq The name 'Bargil was listed as applicable to a 'poisonous black or dark-coloured snake about a metre in length' A knowledge of Iraqi snakes suggests at once that the description applies to *N morgani* alone On the other hand it was never my experience to hear the word used in the field at all I found invariably that a black or dark snake was literally so described as 'Urbid', 'Abiid , or 'Hara Soda', and in Kurdistan 'Mari Rush' However there are more tangible things than a colloquial name common to both

W ægyptia and *N morgani* resemble each other in having the neck slightly dilatable only , in the proportion of the tail to the total length , in being of a uniform coloration with the venter of a slightly paler and flatter shade than the dorsum, the colour being black, purplish, or dark brown , in the distribution and proportions of the head-shields and in the counts of the ventrials, dorsal rows, and caudals In both species six or so of the more proximal of the latter are undivided, while the remainder are divided

They differ, firstly, in that the first named has been recorded only from Egypt and the other only from Arabistan (Persia) and Iraq , secondly, in that the maximum recorded length of *W ægyptia* is 1,280 mm and that of *N morgani* only attains 902 mm , and thirdly, there is the absence of small teeth behind the fangs in *W ægyptia* The first two of these differences signify nothing , the third is vital Of the six specimens of the Egyptian species, I wonder how many of the twelve sets of maxillary teeth have been examined I personally know of one dissection only and the result was quite inconclusive

I shall await the publication of further material, or the opportunity of examining further specimens myself, with interest, and in the meanwhile tentatively suggest that the snakes are conspecific and should stand as *Naja ægyptia* (Lataste)

I give here a table of what characters I have been able to collect concerning the two species —

TABLE I

Species	Length (mm)	Ventrals	Caudals	Pores	Anal	Supralabials		Colour	Tail total Ratio	Remarks
						Number	Into eye			
N. aegyptia (Anderson *loc cit*)	1,187	189	48	25 23	2	7	3-4	Dark brown Belly paler	1 7	
N. aegyptia (Mertens *loc cit*)	1,280	191	45	27 23	2	7	3-4	Dark brown Belly paler	1 7 1	
N. morgani (Mocquard *loc cit*)	902	?	40 to 46	?	2	7	3-4	Dark brown Belly paler	1 6 8	Composite of his five specimens
N. morgani (*A. wilsoni* Wall *loc cit*)	819	?	?	?	?	7	3 4	Uniform black Belly plumbeous	?	
N. morgani (Writer—Mosul)	304	185	46	21	2	7	3-4	Black Belly paler	?	
N. morgani (Writer—Mosul)	635	183	43	21	1	7	3-4	Black Belly paler	1 6 5	
N. morgani (Writer—Baqubah)	675	185	41	21	2	7	3-4	Purplish brown Belly paler	1 7 1	
N. morgani (Writer—Mandali)	736	185	36+	23	1	7	3-4	Black Belly paler		Tail mutilated
N. morgani (Writer—Rutba)	558	182	45	21	2	7	3 4	Black Belly paler	1 6 9	

Naja morgani is thus found from south-western Persia (Arabistan) to Rutba in the Shamiyah Desert via Mesopotamia south of Mosul. If *Naja aegyptia* should prove to be valid the western limit of distribution is Eygpt and the snake may be expected to occur in northern Arabia and the Levant.

As mentioned above, the snake is known to the natives of Iraq as the 'black snake', that is, in Arabic 'Haia Soda', 'Urbid' or 'Abrid', and in Kurdish as

'Mari Rush' As such it is confused with *Coluber jugularis* var *asianus* in its melanotic forms

Specimens were taken in such diverse surroundings as a mud tennis-court, a hole in Mosul Hospital gateway, a bricked water-containing well, a desert track near cultivation, and bare desert A specimen handled freely by several persons hissed angrily but made no attempt to bite

Family VIPERIDÆ

Sub-family *VIPERINÆ*

The Levantine or Blunt-nosed Viper, **Vipera lebetina** Linnæus

This snake was first described by Linnæus (1766) as *Coluber lebetinus*, its provenance being recorded merely as 'Habitat Oriente'

Its distribution is in Europe, the Cyclades and Cyprus, in Africa, the Atlas Mountains of Morocco and Algeria, and in Tunisia, there are no records from Egypt, in Asia it is found in Asia Minor, the Levant, Transcaucasia, Northern Iraq, Persia, Northern Baluchistan, Afghanistan and Kashmir

Boulenger (1920) recorded its occurrence in Iraq from Baghdad and Aushuiu From 1927 to 1930 I examined seven more specimens They came from Sulamainia, Mandali, Mosul, Baqubah, Jebal Hamrin, Qaiagan, and Barazan on the Greater Zab River, all these places being north-east of Baghdad and either in hill country or immediately adjacent to such country No specimens have been recorded from the desert plains south and west of Baghdad This is perhaps not surprising when its absence from Egypt is considered in contrast with its occurrence in and near the Atlas Mountains It appears to inhabit a continuous succession of barren, hilly features from the Atlas Mountains of the west via the Cyclades, Taurus Mountains, Levantine Highlands and Persian Plateau to the north-western mountain masses of India

That it is confined in Iraq to the north-eastern quadrant of the country, pivoting on Baghdad, is also suggested by evidence from two other sources, firstly that of the vernacular names, particularly those referring to its attributes of 'deafness and blindness' and secondly that of the distribution of snake-bite cases considered in conjunction with the distribution of other Mesopotamian vipers

The attributes of the Levantine Viper which characterize it, and distinguish it readily from other snakes occurring in Iraq, are its torpidity by day, and shape, particularly when considered in conjunction with its size and poisonousness To the native who encounters it, generally on barren, gravelly hillsides under stones, it is blind and deaf because, instead of shooting away in a flash of coils, it moves, if at all, slowly and sluggishly Shouts and excitement, stones and sticks, all leave it apparently oblivious, that is, it is deaf and blind Needless to say there have been occasions, when it was stepped on, when only too much liveliness was evinced *C cornutus* and *E carinatus*, the other vipers of Iraq, exhibit the very greatest

activity when disturbed The head and neck of *V lebetina* though viperine in shape, are not so defined as those of *E carinatus*, and do not approach the 'ace of spades' appearance of the cephalic end of *C cornutus* The body, however, relative to the whole animal, is fatter and the tail thinner and more abrupt A large *V lebetina* may be twice the size of the largest of either of the other two species An average adult *V lebetina* is, in Iraq, of a pale ash colour blotched indistinctly with a reddish brown The other two species to a native, as to myself, would convey an impression of yellowishness

In short, any snake described in Iraq as deaf or blind, or deaf alone, is a Levantine Viper If in addition, bigness redness, fatness of body and thinness of tail, and poisonousness are also mentioned, identity is made doubly certain

In discussing the vernacular names applied to this snake it may first of all be mentioned that in Cyprus, where the snake is very common, it is referred to as 'Koufi', a Greek derivation meaning 'deaf' (Anderson, 1898) Further, the 'deaf adder' of the Psalms can be no other than *V lebetina*, the *Daboia xanthina* of Tristram (1883)

An Arab snake-charmer in Mosul in 1929 described to me amongst other local snakes 'Haia Amia', the 'blind snake' This, he said, was blind and deaf, of a brown colour, had a fat body, a tail like a cigarette and sometimes showed its teeth (fangs ?) The village notables of Dohuk further north also spoke of the 'Haia Amia', they said it was blind and deaf, had a fat body and a small tail, was grey with brown marks and was deadly poisonous They said its name in Kurdish was 'Mari Kura' 'Haia Tursha', the deaf snake, invariably claimed to be fat, sluggish and deadly poisonous, I have had described in Mosul, Suwara Tuka area north of Mosul, Erbil, Kirkuk, Qaragan and Khaniqin All these places are in the north-eastern corner of Iraq From the desert plains south and west of Baghdad no descriptions of, or names applicable by any stretch of imagination to, the Levantine Viper (except two given below) came my way in over two years during which was amassed material from such varying sources as natives, students, notables and officials The excepted two names came from Diwaniyah in the Middle Euphrates and were communicated by a native who was careful to impress upon me that these two snakes were only found in the north of Iraq and not in the Diwaniyah area The first of these names was 'Haiat al Farsiyah', the 'Persian Snake', which was said to be big, very poisonous, brown and grey, and found only on the Persian frontier this is very much a true bill for *V lebetina* The other name was 'Arbid al Nahrani', the 'River Snake' It was said to be dark red, poisonous, and found in towns in the north of Iraq It is of interest to mention in connection with this last name that in 1929 Mr J Parlby told me that he had seen travelling downstream in the Diyala River near Qaragan, a snake answering to the description of *V lebetina* The evidence of the vernacular names points definitely to this snake's occurrence in north-eastern Iraq only

I am labouring on the subject of distribution on account of its interest and importance when cases of bite by unidentified snakes are considered

In Iraq, as elsewhere, a man bitten by a snake rarely secures it and often can only describe it as 'a snake' Seventeen examples of such cases, in which the symptoms were those of viperine poisoning are detailed below No fewer than fifteen of them came from this area, where *V lebetina* is common, where two cases of bite in which the offenders were secured and identified as *V lebetina* came from, where no *Echis carinatus* has been recorded from, and from where only two out of ten *Cerastes cornutus* recorded for the whole country have been reported, these two incidentally not being thoroughly authenticated *V lebetina* stands suggested as the culprit The correct isolated significance of this is presumably that *V lebetina* is at any rate very much commoner in the north-east of the country than elsewhere

I know of no literature or data relating to the mutual exclusiveness of viperine species on œcological grounds but possibly there may be a further argument available in this respect

In any case I feel justified in contending that the various facts relating to *V lebetina* mentioned above can mean only one thing, when taken together, and that is, that this species is limited to north-eastern Iraq

The Horned Viper **Cerastes cornutus** (Linnæus)

This species was first described under the name of *Coluber cornutus* by Linnæus (1757) as occurring in the Levant

Boulenger (1915) gave its distribution as 'Soudan and borders of the Sahara, Arabia and Palestine' The snake is indisputably figured on the monuments of ancient Mesopotamia (Koldewey, 1914, and King 1915) In 1920 Boulenger recorded six hornless specimens from Mesopotamia, they were from 'Basra and Shaiba' In 1927 the writer was presented with a fine horned specimen taken at Nebr, near Nasiriyah on the lower Euphrates In 1930 a horned specimen was received from Rutba in the Shamiyah Desert It is notable that these eight records are from flat desert areas north-east of the Euphrates Towards the close of 1929 a Syrian doctor was sent to enquire into a case of sudden death at Dera Gidjnik near Dohuk, north of Mosul He returned satisfied that the man had died twenty-four hours after being bitten by a snake described by the relatives as having horns, being two and a half feet long (recorded in hand spans) possessed of a thick body and with the head broader than the body The snake had been buried The bodies of neither snake nor man were exhumed for examination Natives of Mosul and Dohuk, and further north, questioned by me invariably stated that they had heard of horned snakes but never seen them There is a story in Baghdad about a fatality in the Turkish time caused by a snake with a triangular head, this must have been a *C cornutus*

If the Horned Viper does occur east of the Euphrates it must be very rare It appears to be found mainly in the desert plains west of the Euphrates

Vernacular names for it are numerous Referring to its horns are 'Qarna', 'Um Grun' and 'Haia Bikarun' Its habit of coiling to a flank with its head threatening to the front has secured it the name of 'Um Jenaib' ('Abu Jenaib' is the name applied to the local freshwater crab) A hornless specimen would probably share the following names with *Echis carinatus*, both being of similar appearance, yellowish and poisonous, and moreover both having the habit of making a rasping noise by rubbing their coils together 'Haia Safra', the 'yellow snake', said to be yellow and poisonous and 'Efa' the classical word for viper In Mosul I was told of the 'Fiyah', presumably a corruption of 'Alfiyah', pertaining to 'a thousand' This was applied to a two-horned snake There is a certain amount of folklore about poisonous snakes that live to be thousands of years old So much for the Arabic names In Kurdish there are two names that appear to be applicable 'Mari Zurt' used of a yellow and poisonous snake, and 'Mari Swara' used of a yellow poisonous snake that throws itself at people on horses! In this connection it is interesting to recall that Anderson (1898) attests the saltatory powers of *Cerastes cornutus*

The Saw-scaled Viper, **Echis carinatus** (Schneider)

This snake was first described by Schneider (1801) as *Pseudaboa carinata* Boulenger (1915) gave its distribution as 'desert and sandy districts of Africa, north of the Equator, Southern Asia, from Transcaspia and Arabia to India' Sinderson (1924) recorded its occurrence in the Diwaniyah area of the Middle Euphrates in connection with a snake-bite fatality in 1923 The next year two policemen in Diwaniyah were bitten by two snakes they were handling The snakes were identified as specimens of *Echis carinatus* In 1927 occurred another case of snake-bite in this same area The snake was secured and identified as *Echis carinatus* No records of this snake have come from elsewhere in Iraq and it would appear that the distribution is confined to the sandy desert patches of the Middle Euphrates Apropos of such an isolated distribution it is interesting to note that Wall (1928), discussing its occurrence in India, writes 'it is distributed chiefly in isolated patches where it is frequently very common'

Echis carinatus enjoys all the attributes of the hornless *Cerastes cornutus* Incidentally it may be pointed out that seven out of the ten *C cornutus* recorded above were hornless, and Anderson (1896) in a series of thirteen found no fewer than seven without horns In such circumstances there would be little to distinguish the species (except to the expert) and the following would be characteristic of both Yellowishness, broad head neck defined, fat body, thin tail, sideways coiling with head to the front, slithering under the sand, rasping noise made by rubbing the coils

together, power of jerking (jumping) about, comparative smallness of size and poisonousness

The following Arabic names might be used of it in the Diwaniyah area as they would also of a hornless *Cerastes* 'Um Jenaib', 'Efa' and 'Haia Safra' A native of Diwaniyah once told me he had heard of a snake called 'Sul al Taiyur', that is, 'thin one of the bird', it was brown and powerfully poisonous A characteristic of *Echis carinatus*, if present, is a dark marking as of the four toes of a bird on the head I have no records of this name from other parts of the country

This zoological preamble may be fittingly concluded with a simplified key to the identification of the five poisonous species It is, of course, formulated with careful regard to the characteristics of the non-poisonous species that occur in the country These are *Typhlops braminus*, *Typhlops vermicularis*, *Leptotyphlops macrorhynchus*, *Eryx jaculus familiaris*, *Natrix tessellatus*, *Coluber dahli*, *Coluber diadema*, *Coluber jugularis*, *Coluber navergieri*, *Coluber ventrimaculatus*, *Lytorhynchus diadema*, *Contia collaris*, *Contia coronella* *Contia decemlineata*, *Contia persica*, *Tarbophis fallax iberus*, *Malpolon monspessulana*, *Malpolon moilensis*, and *Psammophis schokari*

Species	Field identification	Clinching characteristics
E schistosa	From the sea or Shatt el Arab Oarlike flattened tail Grey, green, or blue, barred darker Belly paler	Mid body rows 49—66 Ventrals slightly enlarged Scales sub imbricate with faint central keels
N morgani	The only uniform black, purple or dark brown snake in the country No neck and a small eye and stumpy tail (*C jugularis* may have a black dorsum but it has a coloured or white belly, a large eye and a tapering tail)	Fangs, no loreal, third supralabial touching eye and nostril Several of the more proximal caudals single, others divided
V lebetina	An olive, brown, grey or reddish grey snake with a blunt snout, a thin neck, a fat body, a very stumpy tail and no large plates on the head The scales are keeled and the pupil vertical Found particularly under stones in a torpid state by day, in N E Iraq	Erectile fangs Ventrals 147–180, caudals 29—51 divided, dorsal rows 23—27
C cornutus	May have horns 'Ace of spades' head, fat body, thin stumpy tail, yellowish, spotted brown, keeled scales, vertical pupil, no plates on head Rasps coils against each other	Erectile fangs Ventrals 130—165, caudals 25—42 divided, dorsal rows 27—35
E carinatus	As for *C cornutus* without horns, except that the flat triangularity of the head is not so defined, there may be wavy lines instead of spots down the back and there may be a 'broad arrow' or 'birds foot mark on the head Found in Diwaniyah area only as far as is known	Fangs It is the only snake in Iraq with the complete series of subcaudals *undivided*

SNAKE-BITE IN IRAQ

I know of no evidence that suggests any appreciable climatic or economic change in Iraq within historic time, nor does there exist to my knowledge any information suggesting that the ophidian fauna has changed It is therefore of interest to note any references or evidence that may have survived the passage of time, relating to snake-bite in the ancient civilizations of the country

Nerighissar in describing his beautifying of the Temple of Marduk in Babylon speaks of 'eight serpents standing upright which hiss deadly poison' (Koldewey, 1914) Dr R Campbell Thompson was good enough to point out to me three interesting references included in his Assyrian Medical Texts There is 'A prayer to avert the evil of a snake that it should not approach the King', coupled with an apparently magical procedure involving a part of the snake, herbs and certain rites More to the point perhaps is 'If a snake bites a man, thou shalt peel root of cyperus, let him eat it and he shall recover' There is also 'If a snake bites a man he shall drink calendula (?) in beer and recover' Mr C J Gadd of the British Museum very kindly gave me a certain amount of information about snakes in ancient Mesopotamia and mentioned amongst other items 'an evil snake' that might fall upon a man Mr Sydney Smith (late Director of Antiquities, Iraq) supplied me with the very interesting information that the Assyrians had a Sumerian loan-word, Muslahhu, meaning 'washer away' of snakes This would appear to indicate the existence of snake-charmers, and thus suggests that snake-bite was an established danger then, as now

Professional and amateur snake-charmers are found in Iraq to-day In my experience they are ignorant and very lucky They mostly exhibit the harmless Gray's Whip Snake, *C ventrimaculatus*, the commonest and most easily obtained snake in the country Fortunately for them the poisonous snakes are rare in comparison Such a person is generally known as a Sheik (a word of diverse application) in consequence of his believed supernatural powers The Sheik of Case I recorded below was not one of the fortunate ones

I was told in Baghdad and Mosul that a snake-bite is more dangerous if delivered by the snake while on its back A belief is held in Diwaniyah and Baghdad that bites on the ankle are always fatal while those elsewhere are not necessarily so I was told in Baghdad that if the water in which a poisonous snake had been boiled was drunk a partial immunity to poisoning by that type of snake was conferred

The drinking of milk is a popular remedy for snake-bite throughout Iraq In Baghdad I was told that the correct mode of treatment was to give

milk to the patient in great quantities and then have him swung violently by the four limbs till he vomited Dr T H McLeod told me that in Diwaniyah it was customary to shake bitten persons to prevent their sleeping In the Muntafik Liwa, Dr I D Ramsay said it was commonly stated that a bitten extremity should be placed in the newly opened abdomen of a sheep or goat This same belief is held in the Erbil area A snake-stone is another remedy used in the Muntafik Dr T H McLeod informed me that further north, in Diwaniyah, snake-stones are known also They are made from earth mixed with *the semen or vomit of a (or the ?)* snake and when dry are applied to the wound

The town notables of Dohuk north of Mosul, in September 1929 told me that certain of the inhabitants of the neighbouring village of Mungash were held in esteem for treating snake-bite by suction preceded by scarification I gathered it was of the nature of a family craft handed down from father to son Dr T H McLeod in his monthly report for April 1924 from Diwaniyah stated that a local Seyyid (descendent of the Prophet) had a reputation for treating successfully snake-bite cases by suction

To turn to the more sophisticated aspects of snake-bite in the country it is surprising to note that the British Troops during the War had apparently no trouble with snakes, for the Director-General of the Army Medical Service in reply to an inquiry on the subject was good enough to communicate on 22–5–30 that 'the records of the Mesopotamian Expeditionary Force now available do not contain any reference to casualties from snake-bite' The Principal Medical Officer of the Royal Air Force Medical Service in Iraq kindly ascertained for me in 1929 that their available records in the country, which covered the years 1923 to 1929 inclusive, were devoid of reference to snake-bite The Director of the Medical Service, Iraq Army, was able to supply me with a single fatality (Case I below) Search amongst the records of the Iraq Health Service and communications from colleagues, however, gave me a further twenty-four cases

The only literature on snake-bite in Iraq is Sinderson's (1924) interesting paper on an *Echis* fatality This is amplified slightly as Case XX below *

The twenty-six cases recorded as having occurred in Iraq may be taken as covering, say, fifteen years (since 1915, the War period) Even if doubled to allow for cases not coming to notice, the annual incidence in a population of 2,800,000 people is thus shown to be economically negligible However the scientific interest remains It is justifiable then to examine such cases in detail The following table summarizes the analysis —

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* *See* January 1933 number of the *Journal* (In the Press)

Plate XXII

Enhydrina schistosa, the Hook-nosed Sea Snake

(*By Courtesy of the Royal College of Medicine, Baghdad*)

TABLE II

Snake	Total cases	Fatal cases	Mortality per cent	REMARKS
E schistosa	*Nil*	*Nil*		
N morgani	*Nil*	*Nil*		
Probably *N morgani*	2	2	100	Very small series
V lebetina	2	1	50	Average mortality = 33·3 per cent
Probably *V lebetina*	13	4	37	A fair series
C cornutus	*Nil*	*Nil*		
Probably *C cornutus*	2	2	100	A small and doubtful series
E carinatus	4	1	25	Average mortality = 40 per cent
Probably *E carinatus*	1	1	100	Small series
Unknown	2	*Nil*		Possibly *Cerastes* or *Echis* or a *Coluber*
TOTAL	26	11	46	Probably too high an index

POISONING BY *E schistosa*

There are no records from the waters or coasts of Iraq, but the literature is of interest

That on the venom is scanty but Fraser and Elliot, and Rogers (1903–04) stated that the venom was ten times as toxic as that of the Cobra, *Naja tripudians (naja)*, and that its effects were exerted upon the vagus centre, the respiratory centre and the motor nerve endings in the diaphragm The yield, however, was very small Sea-snakes are shy and inoffensive, their mouths are small and their fangs of an apparent insignificance They are only liable to be encountered to any extent by fishermen, would presumably be easily noticed, and would certainly be treated with great respect as are the most harmless snakes by the vast majority of natives It is not to be expected therefore that an examination of the literature will reveal much I have found one case only, that originally recorded by Peal and quoted by Calmette (1908) In this instance a man was bitten by a snake caught in a fishing net at Dhamra in Orissa, was given Calmette's serum when unconscious seven hours later and, reacting favourably, was given more about twelve hours later

(nineteen hours after the bite) He was quite well twenty-one hours after the bite He was bitten on the thumb and his symptoms were, collapse, semi-consciousness, inability to speak, inability to stand, drooping of the eyelids, swelling and stiffness of the finger, and pain in the back of the neck and the lumbar region

I know of no cases of snake-bite that have occurred on the Iraq coast or amongst the local fishermen

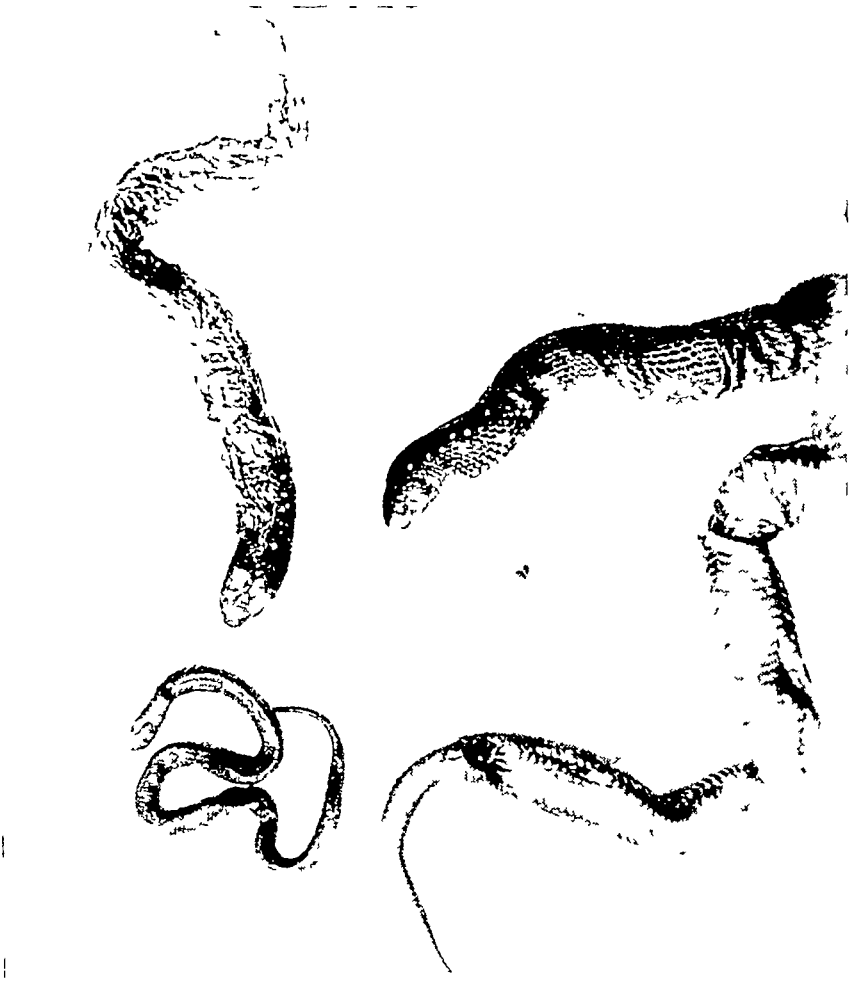
Poisoning by *N morgani*

Nothing has been recorded about the venom of this snake nor have any cases of poisoning ever been reported A fair literature is available, however, relating to its Indian congeners *Naja naja*, the Hooded Cobra and *Naja bungarus*, the King Cobra There is also a certain number of data available respecting its African congeners *Naja haje*, the Egyptian cobra, and *Naja flava*, the Cape cobra

Wall's (1928) review of the literature suggests that the Indian species have venoms of comparable potency The Hooded Cobra, *Naja naja*, discharges possibly ten lethal doses in a single bite, and *Naja bungarus* more in proportion to its much larger size Bites by *Naja naja* are characterized by general weakness, first noticeable in the legs, by drooping of the head and eyelids, loss of voice, loss of power to swallow, nausea and vomiting, and increasing difficulty of respiration, in cases about to terminate fatally the end is often heralded by a convulsion Death, if it is going to occur, often takes place within six hours The reaction at the site of injury is small in comparison with that resulting from a viperine bite Many cases have been well recorded, notably those of Hilson, Richards, and Lamb, quoted by Wall (1928) in his 'Poisonous Terrestrial Snakes of India' The same writer mentions four cases of King Cobra poisoning in which death occurred in a very short time This of course might be expected, the venoms being equally powerful, but the King Cobra being able to discharge much more poison in a single bite

Naja haje has according to Calmette (1908) a venom of much lower potency I know of no fatality having resulted from its bite, but Calmette quotes Professors Keatenje and Ruffer of Cairo as reporting recovery of a thirteen-year old girl at Gizeh, who was bitten by a three-foot Egyptian cobra and brought to hospital in a state of complete collapse, she received Calmette's serum and was convalescent two days later

Epstein (1930) published some work on the venom of *Naja flava*, the Cape cobra I believe the animals experimented on were cats The venom was similar in its action to other Cobra venoms I have only seen a reference to this work however Fitzsimons (1912) quotes a case of death following a bite by a Cape cobra, *Naja flava* The patient, a woman, was bitten on the ankle, the fangs piercing local vessels The woman was blind in five minutes, collapsed within ten minutes and died within three-quarters of an hour



Naja morgani, the Hoodless Cobra
(*By Courtesy of the Royal College of Medicine, Baghdad*)

Monserrat, Schobl, and Guerrero (1920) found that the venom of the Philippine cobra, *Naja naja philippinensis*, was slightly more toxic and slightly less hæmolytic than that of other members of the genus

From the foregoing it would appear that the members of the genus have a characteristic venom which varies in some degree of potency as between species The larger members of the genus, other things being equal, will be the more dangerous, as they can inoculate larger quantities of venom at a bite The remaining factor affecting their degrees of dangerousness to man is aggressiveness Evidence in this respect is difficult to assess *Naja morgani* is, I believe, not of an aggressive nature, for I know of at least two instances in which specimens were handled and made no attempts at biting

I accept the two following cases of death as being ascribable to *Naja morgani*, my reasons for doing so are given with the cases

Case I —*Bite by Unidentified Snake with Fatal Result Mosul Area* Details communicated by the Director, Medical Service, Iraq Army, Baghdad, 1929

A private soldier who was a Sheik and in addition enjoyed the reputation of being able to handle snakes with impunity, a not uncommon popular attribute, whilst on the march, saw a snake and tried to catch it His companions said that the snake was black and that the Sheik said it was harmless Death occurred between six and ten hours later The actual report of the Medical Officer who attested the occurrence is unfortunately very brief and reads to the following effect 'Recruit Sheik Hassan bin Abbas, on the march between Haman el Ali and Hattra, saw a snake and tried to catch it It bit him on the left hand and the whole of the arm became swollen On arrival at Hattra first aid treatment was given and the patient was sent to Mosul in a cart He died on the way at 5 P M on 14–2–23'

Commentary - I believe the snake to have been *Naja morgani* for two reasons Firstly, this is the only black poisonous snake in the country, and 'psychic' death following a bite by a non poisonous black snake may be ruled out fairly in a man who has a reputation for handling snakes, and moreover himself firmly believes not only that he is immune but also that he is handling a non dangerous snake In any case the only other black snake in Iraq, the fierce but harmless *Coluber jugularis* var *asianus* (melanotic form), has not been recorded from the area concerned, all records to date being from Baghdad and the South Secondly, the mode of action of the poison is typically colubrine, that is, paresis of the legs suggested, by the scanty record that he 'was sent to Mosul in a cart' or, in other words, could not walk, and death in from six to ten hours

I have no data regarding the first aid treatment or whether a tourniquet was used The continuance of the march and the jolting journey to Mosul in a cart may have served to accelerate the distribution and fixation of the venom

Case II —*Bite by Unidentified Snake with Fatal Result Khaniqin* Details communicated by Dr Ridge Jones, Civil Surgeon, Khaniqin, in 1930

Salim, a Kurd aged 55, when in the gardens at Khaniqin on the evening of 8–7 29 was bitten on the foot by a snake of which he gave no description Salim was known as an 'old case of asthma' He was treated with 'incision and permanganate, and also with 'injections of stimulants' His symptoms were 'paralysis of legs, vomiting, collapse, and yawning' He died six hours after being bitten

Commentary —This can only be a case of *N morgani* toxæmia The snake has been recorded from Mandali and Baqubah nearby, and there is no other cobra in Iraq Death in six hours and paralysis of the legs, vomiting and collapse are typical colubrine symptoms The yawning is of interest It may have been the accompaniment of the feeling of intoxication, lethargy and laziness which is an early consequence of cobra bite, or it may have been the mode of recording the fact that as the palsy affected the jaw muscles thus conducing to the lower jaw falling away from the upper and exposing

the teeth and tongue (a constant feature of cobra toxæmia), the patient tried to overcome this unnatural state of affairs by summoning up a failing ability to close his jaws by strong volitional control His old asthma may or may not have counted against him A respiratory function below par would presumably lessen his chance against cobra venom the lethal effect of which is exerted mainly, if not almost entirely, on respiratory mechanisms

These two cases suggest that *N morgani* is at any rate as venomous as *N naja* of India Whether or no it is as liable to strike under equivalent conditions cannot be positively stated

Poisoning by *V lebetina*

There is no literature on the venom of this snake and but one record of a case of poisoning (referred to below) A fair amount of work has been done, however, on the venoms of the European members of the genus *V aspis* and *V berus* and on that of the notorious Daboia of India, *V russelli*

The venom yield of *V aspis* is given by Phisalix (1922) as 10 to 25 milligrams, when dry This same writer places the minimum lethal dose for a man in the neighbourhood of 15 milligrams This puts it on a par with *Naja naja* venom for toxicity, and it would be possible for an average snake to administer more than one lethal dose The physiological action of the venom is primarily one of depression to the vaso-motor centre The presence of cytolysins, hæmorrhagin, and hæmolysin is attested by the same author The bite of *V aspis* is said to be more feared than that of *V berus*, and Viaud-Grand-Marais (1880) has recorded that the mortality from its bite in Loire Inferieure and La Vendée was 14 per cent for a series of 370 cases According to Phisalix *V aspis* poisoning is characterized clinically by severe pain at the site of inoculation and tumefaction, this increases and spreads If the patient survives long enough, hæmorrhagic oozing and necrosis of the affected tissue will probably occur Venom transported by the blood may effect this tumefaction in remote parts of the body, witness the case of Viaud-Grand-Marais (1880) in which an infant, bitten on the foot, presented a very early œdema of the face Lymphangitis and adenitis, as might be expected, are salient features Systemically, fainting prostration, abdominal pains, diarrhœa and vomiting, constriction of the throat, cold sweats, thirst, cramps, icterus, weak and fast pulse and feeble respiration may be noted In fatal cases death usually occurs in coma within twenty-four hours Anæmia and chronic cachexia sometimes prolong convalescence (Viaud-Grand-Marais, 1880)

Vipera berus (the adder found in England) has a poison similar in its effect but less potent I have been unable to find data concerning its yield, but in view of its moderate size its yield is probably comparable with that of *V aspis* The toxicity of its venom for guinea-pigs is tabulated by Acton and Knowles (1921) as one-fortieth of that of *V russelli*, so one would expect fatalities to be rare, and even so limited to children or the weakly and senile Taylor (1930), discussing adder bite in England, quotes Leighton (1901) and Stradling (1893) as mentioning five

Vipera lebetina, the Levantine or Blunt nosed Viper
(*By Courtesy of the Royal College of Medicine, Baghdad*)

authenticated fatal cases The two of which details were given were boys of 11 and 4½ In the first case, œdema, general discoloration, and collapse were followed by death in 36 hours Taylor's own cases are of two adults and two children, none were fatal Pain, œdema, discoloration, collapse, cold sweats, diarrhœa, vomiting, nausea, hallucinations, thirst and sleepiness were notable symptoms Phisalix (1922) says that in 216 bites by *V berus* in Germany only fourteen deaths resulted, a percentage slightly less than half that given for *V aspis* cases In England the mortality is surely very much less

The ill-famed Indian representative, *V russelli*, has a fairly extensive literature Acton and Knowles (1921) give the estimated fatal dose for an average man as 42 mg and the approximate dose given at a bite as 72 mg Nearly two lethal doses may thus be given, at the same time a snake probably rarely administers a thoroughly efficient inoculatory strike The mortality from *V russelli* poisoning I have been unable to assess but Wall (1928) says it is frequently fatal to man and quotes two cases, both fatal, Calmette (1908) quotes no cases at all, a point not without significance, and Anderson (1926) and Raju (1926) quote two recoveries Its reputation at any rate is more evil than that of *V aspis* Wall (1928) has summarized the constituents of its venom as a depressor paralysing the vaso-motor centre (Rogers), a depressor to nerve cells generally, a fibrin ferment clotting the blood (Lamb), an anti-fibrin ferment reducing the clotting power of the blood (Cunningham and Lamb), a hæmolysin destructive to red blood cells (Cunningham and Lamb), a leucolysin destructive to white blood cells (Cunningham), a hæmorrhagin destructive of the lining of blood cells (Cunningham), a depressor to cardiac muscle (Lamb), a vaso-constrictor (Rogers), and cytolysins destructive to the local tissues of the wound and also to the cells of the liver, kidney and testes The cases mentioned above as quoted by Wall and recorded by Raju and Anderson differ clinically in no material details from cases bitten by *V aspis* and *V berus*, except in the apparent increased severity of the symptoms Death in the two fatal cases occurred 23½ and 27 hours after the respective bites

I know of no laboratory work on the venom of *V lebetina*, and am aware of only one reference in the literature to a case of poisoning by this species This was reported by Fraser (1929) from Cyprus A Cypriot shepherd at Famagusta on 20-6-29, whilst kneeling to drink from a forest stream, was struck on the scalp by a 'Koufi' (*V lebetina*) He was admitted to hospital eight hours after the bite, semi-conscious, with œdema of the scalp, face, neck and chest Great pain is recorded and both eyes were closed by the tumefaction Deglutition was difficult and speech impossible, apparently owing to the local œdema The pulse was feeble and fast There was no nausea or vomiting He brought up blood and mucus from the throat the next day (24 hours after the bite ?), this lasted for eight hours. The day after, that is the 22nd (48 hours after the bite ?), ecchymoses are mentioned as occurring, in the eyelids, subconjunctivally, in the throat, and on the right side

of the abdomen Further, œdema and discoloration of the penis and scrotum were noted However, by then he could speak and swallow He was ultimately discharged fit A notable feature of this case is that there was no suppuration, despite the rubbing in of permanganate crystals No hæmorrhage is recorded from the nose, gums, bowels or bladder

The literature relating to poisoning from members of the Genus *Vipera* has been plentifully quoted above to show that as with the Genus *Naja*, the individual species possess venoms similar in action but varying in degrees of toxicity Further, the individual species again vary in dangerousness to man by reason of their giving different yields of poison at a single bite None of the species have the reputation for aggressiveness enjoyed by certain of the *Najas*

Two cases of bite by *V lebetina* are detailed below, and thirteen cases of bites by unidentified snakes These latter I consider ascribable to the same species because, firstly, they resemble cases of *Vipera* poisoning and, secondly, they all come from an area where *Echis* is unknown, *Cerastes* extremely rare or unknown and *V lebetina* fairly common There are no other viperine snakes in the country Moreover, in one or two of the cases internal evidence and the story of the patient have given clues as to the snake's identity

CASE III —*Bite by V lebetina with Fatal Result Salahiyah, Jebal Hamrin* M J, an Arab coolie aged thirty, was moving a boulder at Salahiyah in the S Jebal Hamrin in the early morning of 7-5-28 when he was bitten on the right hand by a snake which was secured and subsequently identified by Dr Mills at the Central Laboratory, Baghdad, as a *V lebetina* It was forty inches long and is shown on Plate XXIV A tourniquet and 'first aid' were applied He was brought to Baghdad later by train, a six hours' journey, and was admitted to the Railway Hospital there under Dr Lanzon

On admission (14 hours after the bite) the patient complained of acute pain all over the right arm which was swollen, blistered and gangrenous The punctures were oozing blood The wounds were cauterized with permanganate and calcium chloride, calcium lactate, adrenalin and liquor strychninæ in two minim doses, four hourly, were administered Epistaxis, hæmaturia, and melæna developed The temperature ranged within 97°F and 99°F and the pulse is recorded as not having exceeded 90 Death occurred at 4 45 P M on 9-5-28, about 56 hours after the bite

Commentary —This was the biggest *V lebetina* of the seven specimens I examined It had firm powerful fangs, and appears to have inoculated a large dose of venom in this case It is noteworthy that the accident occurred on turning over a stone Lying under stones is said to be characteristic of this species

Hæmorrhages from the nose, bowel and bladder are more characteristic of *Echis* poisoning cases than of those of the Genus *Vipera*, the venom of the former presumably being richer in its hæmorrhagin content If such is the case a fairly large dose of venom must have been injected, and yet took 60 hours to kill This would appear to suggest that the venom is less potent than that of *V russelli*, which killed in the two cases quoted by Wall (*vide supra*) in 23½ and 27 hours without bleedings from mucous surfaces being noticed Unfortunately I have no information as to how long after the *V lebetina* bite these hæmorrhages occurred It may be that such symptoms would have appeared in the *V russelli* cases had they lived longer The six hours' jolting train journey may have served to distribute the venom more quickly, that is, in a more concentrated form to distant and vital structures, than would have been the case had immobility been secured for the patient immediately after the bite Practically speaking, of course, the victim had to be removed to the nearest hospital with all speed, but the point is none the less of interest

Case IV —*Bite by V lebetina followed by Loss of Tissue and Recovery Mosul* Details communicated by Dr W Corner, Civil Surgeon, Mosul, in 1929

A man was admitted to Mosul Civil Hospital in August 1929 complaining of snake bite on the right index finger He brought the killed snake with him It was identified by me (N L C) later as a *V lebetina* and was twenty four inches long The victim's friend had applied its 'bile' to the wound

The arm was greatly swollen and the end of the finger sloughed Recovery was uneventful

Commentary —The snake was a small one, five feet being not an exceptional size for this species It was therefore probably immature The folk remedy of the snake's bile is interesting I have no note of such treatment being spoken of anywhere else in Iraq or for that matter on any other occasion, but it is noteworthy that Thomson (1920) mentions in his Outlines of Zoology 'a recent discovery, requiring amplification that the bile of a poisonous snake is an antidote to its venom' I have not been able to trace this to its source owing to lack of access to literature In the case under discussion there is nothing to suggest that the abdominal contents of the snake applied to the bite did anything else but facilitate the advent of sepsis

Case V —*Bite by Unidentified Snake with Fatal Result Qaragan, Jebal Hamrin* Details communicated by Dr Lanzon, Medical Officer the Railways Hospital, Baghdad, in 1929

Shortly after midnight of 24/25-1 29, an Arab railway employee, aged twenty eight, was bitten or stung whilst asleep on the back of the right elbow This was at Qaragan in the southern Jebal Hamrin A 'tourniquet and first aid were both used' Later that day he was admitted to hospital in Baghdad after a six hours' train journey complaining of the wound, hæmaturia and fever This would be about 8 to 12 hours after the bite Two punctures were visible on the back of the right elbow which was livid, swollen and oozing blood from the wounds The patient said that his mother had told him they had been inflicted by a scorpion Neither he nor she had seen either snake or scorpion The conjunctivæ and skin were jaundiced, the expression was anxious, the respirations were hurried, the pulse rate was 104 and his temperature was 101°F Potassium permanganate was applied to the wounds On the 29th (96 hours after the bite) the fever was higher, the pulse thready, the jaundice increased, the urine scanty, and there was sloughing at the site of injury On the 30th (120 hours after the bite) there were noted incoherence of speech, rectal incontinence, retention of urine, and marked anæmia A blood film showed a polymorph leucocytosis and many normoblasts Death occurred that evening at 5 P M, 137 hours after the bite Autopsy revealed hæmorrhages into the liver, spleen and kidneys

Commentary —A scorpion may safely be ruled out as there were none of the characteristic muscular contractions and, moreover, the symptoms are positively suggestive of viperine poisoning The distribution of the viperine species in Iraq as mentioned above points to *V lebetina* as being responsible The two punctures are also in favour of the offender having been a viper

Hæmaturia and jaundice at the most within twelve hours of the bite are remarkable symptoms I am unable to rule out pre existing disease from the scanty data available, it is not impossible that the man may have had malaria or bilharzia or both, quite masked temporarily by the graver condition On the other hand, I have seen red blood corpuscles appear in the urine of a man bitten by an unidentified snake in the Sudan (Wad Medani, 1931) twelve hours after the bite, increase in quantity for two days and then completely disappear On the whole, in view of the state of the blood revealed by the examination of the film and the post mortem evidence of the activities of hæmorrhagin, one is inclined to accept the case as one of typical viperine poisoning Incoherence of speech, rectal incontinence, and retention of urine would appear merely to have signified imminent dissolution In this case again, the train journey may have been harmful, and the permanganate may have facilitated the necrosis of the injured area

Case VI —*Bite by Unidentified Snake followed by Recovery Kurdarrah, Jebal Hamrin* Details communicated by Dr Lanzon, Medical Officer, Railways Hospital, Baghdad, in 1929

Pointsman Mohammed Yassin, an Arab, was bitten on the right ankle by a snake on the evening of 4-5-28 at Kurdarrah, near Salahiyah in the southern Jebal Hamrin

Two punctures are recorded as having been noted by eye witnesses His leg and thigh swelled up There was hæmorrhage from the punctures He refused all medical treatment including first-aid A report was received that he subsequently died An inquiry early in 1930 as to the truth of this showed, however, that he was still a happy and healthy employee of the Iraq Railways

Commentary —One is tempted to speculate as to what the result might have been had a jolting tram journey and permanganate treatment followed the bite immediately The symptoms point to a viper as having been responsible, in this area *V lebetina* alone is probable

CASE VII —*Bite by Unidentified Snake with Fatal Result Tuz Khurmatu* I am indebted to Dr F Shaw of Field Headquarters, Turkish Petroleum Company, Tuz Khurmatu, for the details of this case and also for an opportunity of seeing the patient in the later stages

A R, an Arab coolie aged 25, who was in the employ of the Company was walking from Tuz village to the local railway station shortly before seven o'clock on the evening of 24-10-29 He was bare-footed, and whilst crossing some grassland near Tuz gardens he put his foot on something soft and immediately heard a hiss This was at once followed by a prick below the right internal malleolus, accompanied by an immediate diffusion of a burning sensation throughout the right foot He reached the station all right and then lost consciousness This he regained at noon next day (approximately 17 hours after the bite and loss of consciousness) when he found himself in a Seyyid's house in Polkanah, to which he had been removed by friends for treatment There was the usual reading of Koranic excerpts and intonation of prayers He sceptically demanded that he should be taken to the neighbouring dressing station of Suleiman Beg This was done and the Medical Assistant there, after carrying out the conventional incision and rubbing in of permanganate, sent the patient on immediately to the hospital at Field Headquarters, Tuz Khurmatu, where he came under the care and observation of Dr Shaw He was admitted at 3 45 P M on 25-10-29, about 21 hours after the bite

The affected leg was swollen to the knee and of a bluish black colour, it was giving off an offensive odour It was painful and exquisitely tender The radial pulse which was almost imperceptible, had a rate of 110 The temperature was 97°F A thick dry fur coated the tongue The patient was covered with a cold perspiration and vomited continuously Nevertheless he asked repeatedly for water, which had been denied him by his friends on the grounds that it would kill him Under chloroform the initial incisions were enlarged, and several new ones made on either side of the leg below the knee In addition, the lower and inner aspect of the thigh was incised The exposed tissues were almost black and bled freely Hot boric foments were applied to the leg and the patient was returned to bed which was warmed with hot water bottles The patient came quickly out of anæsthesia and shortly after was given small quantities of soda and milk, he did not vomit At 6 P M and 8 P M he was given drugs by mouth, they totalled to tr digitalis minims 10, liq strychnin hydrochlor minims 8, liq adrenalin hydrochlor minims 25 and liq bromochloral one drachm The pulse showed no appreciable improvement and restlessness was present throughout the night, although a ¼ grain of morphia was given at 10 P M The next morning, 37 hours after the bite, the pulse had improved, the tumefaction had decreased, and the leg was a healthier colour, but a new area of discoloration had appeared stretching from the great trochanter to the iliac crest The affected inguinal glands were enlarged, hard, hot, and tender Hot boric fomentations were continued and hot permanganate baths were ordered twice daily in addition The patient was induced to take milk and soda, hot coffee, and a small quantity of toast On the 27th, 60 hours after the bite, constipation was treated with calomel 3 grains, mist alba oz 2, and later an enema A satisfactory evacuation was secured No appreciable further change occurred until the 29th, 108 hours after the bite, when the tendency of the affected tissues to necrosis became defined on the outer aspect of the leg Calcium chloride, 10 grains, t d s, orally was ordered Next day, 132 hours after the bite, chloroform was administered and two fresh incisions were made in this area They bled very freely The administration of calcium orally was continued On the 1st of November, 156 hours after the bite, the patient was weaker and a pint of intravenous saline was given, a rigor followed, after which he settled down and felt much better in a few hours' time On the 3rd, 202 hours after the bite, much slough was removed from the leg and the underlying tissues were observed to have a healthy appearance He was given 10 c c of polyvalent anti streptococcal serum and this was repeated the following day The leg was now treated by the

Carrel Dakin method with eusol On the morning of the 6th, the twelfth day after the bite, a large gangrenous patch appeared above the knee and it became apparent that, in view of the ascent of the infection, amputation ought to be considered Unfortunately the patient's strength did not appear sufficient to enable him to stand such a measure at the moment On the 7th and again on the 8th a pint of Bayliss's gum solution was introduced intravenously, and by the morning of the 9th, 15 days after the bite a marked improvement in the patient's strength was noted and operation decided upon Morphia grain ¼ and atropine grain 1 150 were given and amputation three inches below the lesser trochanter was performed, chloroform anæsthesia was used The patient came well out of the anæsthetic and in the evening at 6 P M said he felt fairly comfortable, his pulse was good He was given a ¼ grain of morphia at 10 P M and then slept till an hour after midnight when he awoke in a state of great restlessness Three quarters of an hour later he collapsed and died This was on the sixteenth day after being bitten

Commentary—There is little doubt that the snake concerned was *V lebetina* Firstly, Tuz is in the area where *V lebetina* is common and where the other two vipers of the country apparently do not occur Secondly, the patient heard a hiss Now, whereas members of the Genus *Vipera* do hiss, members of the Genera *Cerastes* and *Echis* do not They make a thoroughly different and characteristic rasping sound instead by rubbing their coils together The symptoms of the case are undoubtedly viperine and the evidence points to *V lebetina*

Seventeen hours' unconsciousness is of interest The patient's haste (i e, muscular and circulatory activity) to reach the railway station probably gave the venom optimum conditions for efficient action by rapid diffusion throughout the victim's system In the initial stages of this case the systemic effects, vaso motor depression, vomiting, cold sweats, hypothermia, dominated, or, at any rate, were more urgent than, the local symptoms of œdema, pain and discoloration Usually in viperine cases, particularly if fear and concern induce immediate cessation of all activity, the reverse is the case

On the morning of the 26th, 37 hours after the bite, the patient's resistance appears to have conquered the initial physiological effects of the poison, but from then on to the end, sapped by the insidious leucolytic and anti bactericidal actions of the venom, a losing fight had to be waged with an ever increasing toxæmia, derived from the necrotic sites on the leg and probably from injured visceral cells also A septicæmia may or may not have been present Degenerating liver and kidney cells, in addition to providing toxins as mentioned, would of course negatively count against the patient through being unable to discharge their normal functions The apparent improvement following the intravenous administrations on the three days preceding death may have been due to the largely mechanical effects of increase in the volume of vital fluids, and at the same time dilution of the concentration of toxic content One may assume that the heart musculature after sixteen days' contact with a blood, robbed of its white cells, with its red cells invalid and lessened and with its plasma altered to a lesser or greater degree by venom constituents, products of necrosing tissue, bacterial toxins, and abnormal products of metabolism, would have difficulty in meeting the demands of a major operation Still the patient would undoubtedly have died otherwise, and operation gave him the remote chance

This case is of the 'textbook' type and may be taken as characteristic of poisoning by *V lebetina* One's feeling is that the patient might have had a better chance had he not hastened on to the railway station Slower absorption of the venom inoculated might have enabled the body to deal with it more successfully, but the muscular contractions involved in walking to the station must have quickly distributed the venom in its highest possible concentration A policeman may tackle three ruffians in succession with difficult success, but if they make a combined onslaught upon him, his chances are scarcely worth backing

Case VIII—*Bite by Unidentified Snake with Fatal Result Rowanduz Area* Details communicated by Dr Ridge Jones, Civil Surgeon, Khanqin, in 1929

When in the Kalashin Pass three days north east of Rowanduz in Kurdistan in July 1923 he came across a case of snake bite The altitude was about 8,000 feet above sea level and there was snow on the ground This was well above the tree line The patient was a male Kurd aged about 40 He

gave no details of the accident, except that he had been bitten by a snake about two weeks before No tourniquet had been used nor had the bitten area been incised.

There was extensive sloughing of the hand and the whole arm was in a septic state There was no history of hæmorrhage and there was no jaundice when the man was seen Dressings were left with him but he died shortly afterwards

Commentary —The snake must have been a *V lebetina* for *Echis* and *Cerastes* do not occur in this area, being characteristically found in the sand of desert plains, whereas *V lebetina* is found in mountainous country from the Atlas to Kashmir The symptoms are typical of *Vipera* poisoning also A height of 8 000 feet above sea level is worthy of note The absence of hæmorrhages and jaundice and the interval between bite and death suggests that the dose of venom inoculated was sub lethal as regards its direct physiological action Death here was due as in so many of these fatal bites by members of this genus, to the secondary toxæmia and possibly septicæmia following necrosis of infected tissues

CASE IX —*Bite by Unidentified Snake followed by Loss of Tissue and Recovery Khanqin* Details communicated by Dr Ridge Jones, Civil Surgeon, Khanqin, in 1929

Hassan Ali, a Persian boy aged 11, was bitten on the foot by a snake in the early morning of 18-5-27 at Khanqin

He was admitted to Khanqin Civil Hospital and the wound area was treated with the conventional incision and permanganate He is recorded as having been 'wandering in his mind for five or six days' Cellulitis subsequently developed and was followed by extensive sloughing He was ultimately discharged after three months in hospital

Commentary —Again the snake must have been *V lebetina* on the grounds of distribution and symptomatology The dose of venom inoculated must have been small, for the patient was a boy of fourteen and apparently only had a serious fight with the secondary sepsis The mental symptoms are of interest, and recall the hallucinations mentioned by Taylor (1930) as occurring in a case of poisoning by *V berus* in England

CASE X —*Bite by Unidentified Snake followed by Recovery Khanqin* Details communicated by Dr Ridge Jones, Civil Surgeon, Khanqin, in 1929

Nazli Ali, a Persian girl aged 18, was bitten on the foot by a snake on the night of 16-10-27 in Khanqin Gardens

She was admitted to the Civil Hospital, Khanqin, and the leg which became septic was treated with incisions and drainage She had no constitutional symptoms and made an uneventful recovery

Commentary —Arguing from distribution and symptoms again, the offending snake must have been a *V lebetina* If so, the dose inoculated was very small Possibly, as this bite occurred at night and the species is a nocturnal feeder, the glands may have been more or less empty when the bite was inflicted or it may have been that the snake was immature, or again the 'strike' may have been inefficiently delivered

CASE XI —*Bite by Unidentified Snake followed by Recovery Kirkuk* Details communicated by Dr W Corner, at one time Civil Surgeon, Kirkuk, in 1929

He remembered a harvester bitten on the foot, near the village of Bedawa in the Kirkuk area, in 1926

The patient was seen four hours after the bite The leg had been ligatured There was great swelling at the site of injury accompanied by much pain Suppuration followed The patient ultimately recovered

Commentary —Kirkuk is in the *V lebetina* area and the symptoms are typical

CASE XII — *Bite by Unidentified Snake followed by Chronic Necrosis Kirkuk* Details communicated by Dr W Corner in 1929

He remembered a case of snake bite near Kirkuk The bite was on the foot and a chronic form of necrosis resulted Amputations were carried out at successive levels as the wounds always refused to heal X ray examination revealed marked osteoporosis of the bones The subsequent fate of the patient is unknown

Commentary —The result of the bite suggests a viper, and the locality, *V lebetina* The osteoporosis is an interesting illustration of the local damage that viperine venom may effect by devitalizing tissues For such rarefaction to take place a markedly chronic infection must have been present, and this being the case it would appear that the body takes a long time to regain its normal standard of defensive reaction to infection Unfortunately no dates were obtainable to amplify this record

Case XIII —*Bite by Unidentified Snake followed by Recovery Sulamania* Details communicated by Dr W Corner in 1929

Dr Corner further remembered a case from near Sulamania in Kurdistan The patient had been bitten on the finger and a ligature had been applied for some time The case was seen several days after the bite There was some gangrene round the site of injury and ultimate recovery

Commentary —*V lebetina* is the only viper that has been recorded from this area The gangrene may or may not have been influenced by the ligature

Dr Corner had long since left Kirkuk area when he kindly gave me the notes on the above cases, he was insistent that he spoke from memory and was only prepared to state facts of which he was certain Their lack of detail is thus notable but in one way is welcome, for it means that what facts are mentioned are doubly certain of being correct The three cases are interesting additions to the series

Case XIV —*Bite by Unidentified Snake, followed by Loss of Tissue and Recovery Mosul* Details communicated by Dr Sati of the Mosul Civil Hospital, in 1929

In the spring of 1914 a man came into Mosul Civil Hospital having been bitten on the thumb by a snake A fang was embedded in the wound, it was removed Gangrene followed, the thumb sloughed off, and the man ultimately recovered

Commentary —The symptoms are viperine The fang of a viper is long, delicate, and moveable and as such is infinitely more likely to be torn out in striking than is the shorter, stronger and firmly fixed fang of a cobra Of the three vipers in the country *V lebetina* is the only probable one It is quite common in this area, in which *Echis* does not occur and *Cerastes* if it does occur is extremely rare Sloughing is here again found, typical of poisoning by the Genus *Vipera*

Case XV —*Bite by Unidentified Snake with Fatal Result Mosul* Details communicated by Dr Sati of the Mosul Civil Hospital, in 1929

In July 1929 a man was carrying a partridge in a cage some distance outside Mosul when he saw a snake with a fat body and a short tail, and tried to catch it He was bitten on the thumb, went a few paces and then collapsed He died three hours later

Commentary —*Vipera lebetina* is suggested by several things Its rival for the honours here is *Naja morgani* They both have short tails, but no one would derive from the cobra the impression that it was fat bodied, whereas this would be precisely their impression of the viper Again, few people, hampered by carrying a cage, would think of giving chase to a quick moving cobra, but the sluggishness of *V lebetina*, the 'deaf and blind' snake, would be just the very factor inducing a venturesome spirit to secure it *Echis* and *Cerastes* may be ruled out as in the previous case Death in three hours is more typical of cobra poisoning, but by no means untypical of viperine poisoning when a large dose is injected Immediate collapse as happened in this case, however, seems to me to clinch the diagnosis as one of viperine poisoning The viperine depressors to the vaso motor centres (Rogers) and to cardiac muscle (Lamb) would account for this syncope without any difficulty, whereas cobra venom contains cardio tonic elements (Brunton and Fayrer, Fraser and Elliot) which give the case of cobra poisoning his sturdy pulse and effectually provide against an initial collapse

Case XVI —*Bite by Unidentified Snake followed by Recovery Mosul* Details communicated by Dr I D Ramsay, Civil Surgeon, Mosul, in 1930

An adult Arab, Jassim ibn Yassin, was bitten on the calf muscles of the right leg at Khorsabad (Dur Sharrukin) at 1 P M on 3-4-30 The snake, which escaped, was about three feet long and was light coloured Within a few minutes a tourniquet was tightened above the knee, the wound was scarified and strong permanganate solution was applied

The patient was admitted to the Mosul Civil Hospital, 2 hours after the bite He complained of faintness, and severe pain in the right leg The pulse was 90 and of fairly good volume and tension

The pupils were normal The leg was swollen up to the knee Brandy was administered, carbolic tonents were applied to the injury and the tourniquet was removed The next morning (20 hours after the bite) the patient complained of an increase of pain in the leg Tumefaction had reached mid thigh, and there was considerable discoloration above and below the knee This condition persisted until the fifth day Owing to the swelling, pulsation of the dorsalis pedis could not be appreciated, but the limb remained warm Fomentations were continued and the leg was kept covered with antiseptic dressings On the fifth day (116 hours after the bite) the pain abated and the swelling commenced to subside After another five days (10 days after the bite) the patient was discharged with his leg still slightly swollen but quite functional There was a mild fever not rising above 100°F for the first four days

Commentary—The identity of the snake rests between *Vipera lebetina* and *Cerastes cornutus* The former is fairly common in this area, and the latter if it occurs at all is very rare, all specimens taken in Iraq having come from the Euphrates valley to the South and West Both snakes are light coloured but whereas three feet is an average size for a Levantine Viper the Horned Viper rarely attains twenty inches and has not been recorded as exceeding thirty

Clinically the case is typical of *Vipera* poisoning

CASE XVII—*Bite by Unidentified Snake followed by Recovery Mosul* Details communicated by Dr I D Ramsay, Civil Surgeon, Mosul, in 1930

Hindo Oshema, an Assyrian mountaineer aged 28, was travelling in the hills about fifteen miles north of Mosul, when he was bitten on the right index finger by a snake This was in the dark about 9 o'clock on 19-4-30 His description of the snake was rather staggering 'It was partly coiled and the erect portion was as tall as a man The body was as thick as a man's wrist When the snake struck at him it knocked him down'

He was admitted to the Mosul Civil Hospital on the morning of 24-4-30, that is, 110 hours after the bite The finger and hand which had been scarified, showed marked sepsis and were discharging offensively The arm was swollen to the shoulder Treatment by permanganate baths and carbolic fomentations was instituted The temperature was of the septic type ranging up to 105°F On 26-4-30, 130 hours after the bite, the outlook was unpromising However, the patient ultimately recovered

Commentary—The patient's description of the snake, taken at its face value, conjures up the truly startling vision of a hamadryad or a python! The recorded distribution of such snakes does not of course permit of the possibility being entertained At the same time I have heard, always however at second or third hand, of the existence of pythons in Kurdistan I know of no records from Persia but have often wondered if the jungles between the Elburz Mountains and the Caspian Sea (500 miles or so from Northern Iraq) might not contain some surprising snake species Tigers and leopards are found there *V lebetina* is common in Kurdistan and specimens may reach five feet in length The symptoms of the case suggest a viper It would have been interesting to know how the victim received the bite on the finger, what he was doing at the time, etc There is, however, an explanation of the fabulousness of the description if the snake really was a *Vipera* Hallucinations in a case of *V berus* poisoning have been mentioned above (Taylor), and wandering in the mind for five or six days in a case of poisoning by a snake that was almost certainly a *V lebetina* (Case IX above) The patient described the snake on the fifth day after the bite and possibly his mental state coloured his description At the same time, unacceptable though it be, the details provide a surprisingly perfect picture of a strike by a hamadryad Clinically the case is typical of *Vipera* poisoning, complicated in the usual way with severe sepsis

Fifteen cases of bites by *V lebetina* or by unidentified snakes acceptable as *V lebetina* have been described, of these five were fatal The mortality rate for this species in Iraq is thus placed at 33⅓ per cent This means that the species ranks above *V aspis* in dangerousness to man and may be less dangerous, as dangerous, or more dangerous than *V russelli* The five fatal cases exemplify four ways in which poisoning by this species (or genus) may lead to death Case III illustrates a degree of poisoning in which the dominating symptoms were hæmorrhages and shock, that is, the lethal result was ascribable largely to hæmorrhagin, hæmolysin and anti fibrin ferment The patient thus not

only lost the badly needed support of much of his vital fluid but dysfunction of visceral organs was acutely induced by the deleterious mechanical and chemical actions of the intra visceral hæmorrhages Such poisoning may be termed a 'cytolytic' type of which death in 60 hours is characteristic Case V illustrates what may be called the vitiatory type in which death occurs largely as a consequence of functional impairment of vital tissues rather than by reason of their more radical lysis This would mean a milder degree of poisoning than in the cytolytic type, and the presence of a marked pyrexial response suggests that the action of the venom was initially not sufficiently severe enough to prevent the body's natural defences from functioning to a fair degree This 'vitiatory' type may be said to terminate life in from four to six days Case VII illustrates the 'septic' type of termination in which, superimposed upon a certain amount of cytolysis and vitiation, is bacterial infection and further poisoning by necrosing tissue Death in about a fortnight would appear to be characteristic Case VIII illustrates the same type of death Case XV may be spoken of as a 'depressive' type in which death is due to vaso motor and cardiac depression, the dominating venom elements being the central vaso motor depressor of Rogers, and the depressor to cardiac muscle of Lamb Such being the case, the dose of venom inoculated must have been large Death in a few hours, in this case three, would appear to be characteristic There remains a further type of death from *Vipera* poisoning suggested by laboratory research, but as far as I know unillustrated by any clinical record Lamb has described a fibrin ferment in *V russelli* venom which clots the blood It is present in a small proportion relative to other venom constituents and is generally masked in moderate doses by their counter effects In very large doses, however, it causes intravascular clotting and death in convulsions almost immediately Wall (1928) is of the opinion that *V russelli* is not big enough to inoculate sufficient venom at a strike to secure this effect in human beings This may reasonably apply to *V lebetina*, but it is not inconceivable that inoculation by a full grown snake under optimum conditions in which the fangs enter vessels or a vessel, might be followed by this 'thrombotic' type of death

Theoretically there are thus five types of death, depending for their nature, other things being equal, upon the dose of venom inoculated They may be graded as the 'thrombotic type' with death almost at once, the 'depressive type' with death in a few hours, the 'cytolytic type' with death in from one to three days, the 'vitiatory type' with death in from four to six days, and the 'septic type' in which death results in about fourteen days There would appear to be a period of dangerous false security pending the onset of hæmorrhage (in cases where sufficient venom has been inoculated) and in those surviving the sixth day, a period of a week or so pending a possible ultimate surrender to sepsis In *V lebetina* poisoning the appearance of hæmorrhages from mucous surfaces which apparently may appear as early as twelve hours after the bite, means a serious outlook, it is an indication that a large dose of poison has been absorbed and fixed

(*To be concluded in the January* 1933 *number of the* Journal —Ed)

References —*See* end of paper in January 1933 number of the *Journal*

FURTHER RESEARCHES INTO THE TREATMENT OF CHRONIC BENIGN TERTIAN MALARIA WITH PLASMOQUINE AND QUININE

BY

MAJOR O D JARVIS, O B E, R A M C,

Malaria Treatment Centre, Kasauli

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THIS note is published in order to place on record the results of further efforts at the Malaria Treatment Centre, Kasauli, to discover the minimal effective dose of plasmoquine in combination with quinine in the treatment of chronic benign tertian malaria

Sinton, Smith and Pottinger (1930) recorded results obtained at this Centre with plasmoquine 0 04 gramme plus quinine grains 20 daily for 21 days, the relapse rate in 44 cases so treated being 8 per cent during an observation period of 8 weeks after completion of treatment They remarked that 'the occurrence of even a few toxic cases amongst our population suggests that even although the duration of treatment may be correct, yet the dosage may be too high for general use' They therefore suggested that further experiments should be carried out with a dose of 0 03 gramme plasmoquine daily over periods of 14 and 21 days

Early in 1930, Major P J Ryan, M C, R A M C, who was then in charge of the Malaria Treatment Centre, commenced a course of treatment, which was labelled for convenience of reference, Treatment 'C' This consisted of plasmoquine 0 02 gramme plus quinine grains 10 in the morning and plasmoquine 0 01 gramme plus quinine grains 10 in the evening (i e, plasmoquine 0 03 gramme plus quinine grains 20 daily) for 21 days, both drugs being administered orally

The routine procedure adopted was similar to that already given in detail by Sinton (1926) The patients were all young British soldiers who had relapsed in spite of the usual anti-malarial treatment in the plains After arrival at Kasauli they were subjected to weekly blood examinations by the thick-film method, and in no case was specific treatment begun, unless *P vivax* was detected in the peripheral blood smear immediately before commencement of treatment

Preliminary purgation with calomel (grains 3) and magnesium sulphate was carried out in every case In those treated in 1930 (75 cases), a daily early morning dose of magnesium sulphate was also given throughout the course of treatment and, in addition, 1 grain of calomel on two or three consecutive evenings for two periods, usually about the 9th and 15th days of treatment In those treated by me in 1931 (31 cases) the routine administration of calomel and magnesium sulphate (except for the preliminary dose) was discontinued and a dose of magnesium sulphate prescribed only when a tendency to constipation appeared to indicate it In 1930 the quinine was given in the form of the sulphate in solution, along with citric acid and magnesium sulphate, as recommended by Sinton (1926*a*), while in 1931 the bihydrochloride was used in simple solution in water

After completion of treatment all cases were observed for a period of at least 8 weeks, thick blood films being examined weekly

Results of treatment in the prevention of relapse

Series I —In 1930 75 cases of benign tertian malaria (relapse) were given Treatment ' C ' (plasmoquine 0 03 gramme plus quinine grains 20 daily for 21 days) and were observed for at least 8 weeks after completion of treatment (average 10 7 weeks) Six of the cases relapsed during this period, giving a relapse rate of 8 per cent

Series II —In 1931 a further series of 31 cases was given the same course of treatment as in Series I

No relapses occurred during an average observation period of 12 7 weeks

Adding Series I and II together, 106 cases were treated with plasmoquine 0 03 gramme plus quinine grains 20 daily for 21 days, with a combined relapse rate of 5 7 per cent

This must be considered highly satisfactory, when it is remembered that the relapse rate in this Centre with quinine alone was 68 per cent (Sinton and Bird, 1929) and with plasmoquine alone 22 7 per cent (Sinton and Bird, 1928) With plasmoquine 0 04 gramme and quinine grains 20 daily for 21 days the relapse rate was 8 4 per cent (Sinton, Smith and Pottinger, 1930) so that the reduction of the dose of plasmoquine from 0 04 to 0 03 gramme daily in the 21 days' course has not reduced (but rather increased) the efficacy of the treatment

In August 1931, the Director of Medical Services, India, recommended that Treatment ' C ', as defined above, be adopted as the standard routine treatment for benign tertian malaria in all Military Hospitals in India It was then thought that it would be valuable to determine at this Centre whether equally good results could be obtained with a yet smaller dose of plasmoquine (either daily or total) It was, therefore, decided to test two new courses of treatment, viz ,

Treatment ' F ' plasmoquine 0 01 gramme plus quinine grains 10 twice daily for 21 days (i e , the same period as in Treatment ' C ' but a smaller dose of plasmoquine)

Treatment 'J' plasmoquine 0 02 gramme plus quinine grains 10 in the morning and plasmoquine 0 01 gramme plus quinine grains 10 in the evening daily for 14 days (i e , the same dose of plasmoquine as in Treatment 'C' but for a shorter period)

It should be noted that the total dose of plasmoquine for the whole course is the same in both 'F' and 'J', viz , 0 42 gramme

Series III —Ten cases of benign tertian malaria (relapse) were given Treatment 'F' (plasmoquine 0 02 gramme plus quinine grains 20 daily for 21 days) All were observed for at least 8 weeks (average 12 2 weeks) after completion of treatment, and no relapses occurred

Series IV —Ten cases were given Treatment 'J' (plasmoquine 0 03 gramme plus quinine grains 20 daily for 14 days) All were observed for 12 weeks after completion of treatment without relapse

Although the numbers of cases in Series III and IV are small the results are at least encouraging, and it would seem as if the smaller dose of plasmoquine would prove as effective as that given in Treatment 'C' If further experience confirms this, then the 14 days' course of plasmoquine 0 03 gramme plus quinine grains 20 daily might well be adopted by the Army as the standard treatment in place of the present 21 days' course, and it would be valuable to ascertain if the dose of plasmoquine might be reduced to 0 02 gramme daily for 14 days, without markedly affecting the efficacy of the treatment

The following table shows the comparative results obtained in this Centre with various forms of treatment in chronic benign tertian malaria —

TABLE

Treatment	Daily dose × days		Total cases	Relapse rate, per cent
	Plasmoquine, gramme	Quinine, grams		
Quinine sulphate*	*Nil*	30 (max) × 21–56	667	68 0
Plasmoquine†	0 03 × 28	*Nil*	22	22 7
Plasmoquine and Quinine‡	0 04 × 21	20 × 21	44	8 4
'C' (1930)	0 03 × 21	20 × 21	75	8 0
'C' (1931)			31	0 0
'C' Total			106	5 7
'F'	0 02 × 21	20 × 21	10	0 0
'J'	0 03 × 14	20 × 14	10	0 0

* Sinton and Bird, 1929 † Sinton and Bird, 1928 ‡ Sinton, Smith and Pottinger, 1930

Toxic effects of plasmoquine

Any toxic effects which may be attributable to plasmoquine have been small in frequency and almost negligible in degree In Series I and II (106 cases treated with plasmoquine 0 03 gramme plus quinine grains 20 daily for 21 days) slight cyanosis was noted in 3 cases, and slight and transient albuminuria in 5 cases A few cases complained of abdominal pain which, however, was never severe

In Series III and IV no toxic symptoms were observed

Although, therefore, with a dose of 0 03 gramme plasmoquine daily for 21 days, toxic symptoms are comparatively rare, the mere fact that slight cyanosis may occur makes it advisable that we should ascertain whether a yet smaller dose would be equally effective, and could be given without fear of toxic manifestations

With regard to this latter point it is of interest to note that all malaria convalescents attending this Centre (some 250 in 1930 and 150 in 1931) were given a course of plasmoquine and quinine before being returned to their units after completion of the full observation period, whether or not they had previously relapsed and undergone one of the courses of plasmoquine and quinine under test This course consisted of plasmoquine 0 02 gramme plus quinine grains 20 daily for 10 days in 1930 and for 14 days in 1931, and was administered to the men as out-patients while carrying out their ordinary duties (including games) No toxic symptoms whatever were noted, and no complaint of abdominal pain was made

It is believed, therefore, that a course of plasmoquine 0 02 gramme plus quinine grains 20 daily for 14 days could be given with safety and without special supervision to patients of the same physique as young British soldiers, and that it would be useful to determine the comparative efficacy of this course of treatment in the matter of 'cure'

Conclusions

(1) In the treatment of chronic benign tertian malaria the combination of plasmoquine and quinine in a dosage of 0 03 gramme of the former and grains 20 of the latter daily for 21 days gives results as good as (if not better than) a dose of 0 04 gramme plasmoquine daily with the above dose of quinine over the same period, and seldom gives rise to toxic symptoms which, when they do occur, are comparatively mild

(2) It would appear that equally good results as to 'cure' are obtainable by the exhibition of plasmoquine 0 02 gramme daily for 21 days, or of 0 03 gramme daily for 14 days, in each case with quinine grains 20 daily, as have been obtained with plasmoquine 0 03 gramme plus quinine grains 20 daily for 21 days The risks of toxic effects in either case are considerably reduced

(3) A dosage of plasmoquine 0 02 gramme in combination with quinine grains 20 daily for 14 days can be given to young British soldiers, who are taking a moderate amount of exercise (work and games) without any fear of toxic manifestations

My thanks are due to the Director of Medical Services in India and to the Officer Commanding, British Military Hospital, Kasauli, for the facilities which they have placed at my disposal for carrying out this investigation, to the Indian Research Fund Association for a substantial grant towards the expenses incurred and to Major A E Richmond, O B E, R A M C, for allowing me to publish the statistics of the work carried out in 1930

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Idem (1929)	*Ibid*,	**16,** No 3,	pp 725–746
Sinton, J A, Smith, S, and Pottinger, D (1930)	*Ibid*,	**17,** No 3,	pp 793–814

The experiment was continued for 235 days by which time all the animals kept in darkness had died, while of those exposed to the sun six remained alive and were then killed by drowning The chief causes of death were pneumonia and urinary calculus

Results of the experiment

The results of the experiment were, from the point of view of the thyroid gland, as follows —

(1) The average daily increase in body-weight in the animals exposed to the sun was nearly twice that of those kept in darkness 0 37 g in the former and 0 20 g in the latter

(2) The average duration of life was longer in those exposed to sunlight— 165 as compared with 141 days

(3) Two cases of thyroid enlargement occurred amongst the 18 animals kept in complete darkness the glands of the remaining 16 were not enlarged Of the two enlargements one was a true goitre, the size of the gland (i e, its weight proportionate to that of the body) exceeding the normal mean by more than 2 5 times the standard deviation from that mean The other enlargement was slight and probably not significant, its size exceeding the normal mean by only once the standard deviation (McCarrison and Madhava, 1932) The incidence of true goitre was, thus, 5 5 per cent

(4) Four cases of thyroid enlargement occurred in the group exposed to the midday sun, the gland was of normal size in the remaining 13 animals * Of the four enlargements one was slight and probably not significant, while three were true goitres, whose size exceeded the normal mean by more than 2 5 times its standard deviation The incidence of true goitre was thus 17 6 per cent, a significant increase over that in the group kept in darkness

(5) The mean size of the enlarged thyroids was 18 35 mg per 100 g of body-weight in the rats kept in darkness as against 25 6 mg in those exposed to the sun Not only, therefore, was the incidence of true goitre greater in the latter group but the size of the goitres was greater

(6) Five estimations of the urinary excretion of iodine were made in five of the animals exposed to the sun, the mean of these estimations was 96γ of iodine per litre of urine In five other rats, fed on the same diet but kept in a place into which direct sunlight did not penetrate, the mean of five estimations was 48γ It is to be noted that a higher incidence of goitre and a greater size of the enlarged thyroids occurred in the group excreting most iodine

* One animal died on the 19th day of the experiment, it is consequently excluded

(7) The diet used in this experiment was favourable to goitre-production As judged by the urinary excretion of iodine, this favouring influence was not the result of insufficient ingestion or absorption of iodine The diet was markedly deficient in vitamins A and C, deficiencies favourable to goitre production (McCarrison, 1927, 1928 and 1930) The iodine content of one of these goitres was 0 005 per cent of the fresh gland as compared with the normal content of 0 018 per cent in the thyroids of well fed rats in this Laboratory It would seem, therefore, that while iodine was ingested and absorbed in sufficient quantity the thyroids of certain of the animals were unable to utilize or to store iodine normally Further evidence is thus provided of the fact that impairment of the functional efficiency of the secretory epithelium of the thyroid gland, brought about by certain dietary deficiencies, may conduce to goitre even in the presence of a sufficient intake and absorption of iodine (McCarrison, 1927) It is to be emphasized that the diet is not of itself the cause of the goitre, it renders the gland susceptible to the action of some unknown, positive goitrogenic agent

(8) In this experiment darkness did not exert any action on the thyroid gland, certainly none that was favourable to goitre-production On the other hand, the incidence of goitre and the size of the goitres were greater in those exposed to sunlight, that is to say, in those whose intake of vitamin D was the greater The tendency, not strikingly significant, of vitamin D [administered as radiostoleum (B D H)] to increase the size of the thyroid gland in deficiently-fed rats has been recorded in another place (McCarrison and Madhava, 1932)

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THE EFFECT OF INSANITARY CONDITION ON THE THYROID GLAND AND OTHER ORGANS OF THE BODY

BY

COLONEL R McCARRISON, CIE, KHP, IMS,*

Nutritional Research Laboratories, Pasteur Institute, Coonoor, S India,

AND

K B MADHAVA, MA, AIA,*

Professor of Statistics, University of Mysore

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IN a recent publication (McCarrison and Madhava, 1932) it was shown that while insanitary condition did not cause goitre in albino rats fed on a superior diet consisting of whole wheat flour (*chapatti*), sprouted *gram* (legume), butter, raw carrots and cabbage *ad libitum* with fresh milk in abundance and meat with bone occasionally, it did cause goitre when they were fed on an inferior diet consisting solely of vegetable foods—cabbage, whole wheat and cholam (*Andropogon sorghum*)

It has been shown also (McCarrison, 1931*a*) that cabbage, which formed the principal constituent of the inferior diet above referred to, may contain a goitrogenic agent whose potency varies from season to season Recent observations have indicated that while, in this locality, this potency may be great during and after the rains it may be slight or nil during the dry season, it would seem to be associated in some way with rainfall

Effect of rainfall and of season on the goitrogenic potency of cabbage

Rabbits were fed during different months of the year on a diet of fresh, raw cabbage and water for a period of 56 days † At the end of each experiment they were killed by air-embolism and their thyroid glands removed and weighed The size of the gland relative to that of the body—the 'r'-value, obtained by dividing

* The first author is responsible for the text of this paper and for the experimental material with which it deals, the second for the statistical examination of this material

† In all experiments of this kind the cabbage used must be perfectly fresh otherwise the animals lose weight and exhibit an increased susceptibility to infection, chiefly of the lungs The addition of dried yeast to the cabbage diet helps in the maintenance of body weight and, if anything, tends to enhance the goitrogenic potency of the diet

the thyroid-weight by the body-weight (McCarrison and Madhava, 1932)—was then determined for each animal and the mean value in each experiment calculated These mean values are set out in Table I against the total rainfall for the months in which the experiments were carried out

TABLE I

Showing the total rainfall and the size ('r') of the thyroid gland at different months of the year in rabbits fed on a diet of fresh, raw cabbage and water

Number of experiment	Experiment carried out during the months of	Total rainfall in inches	Mean 'r'-values of the thyroid gland mg per 1,000 g of body weight
I	November and December	39 5	749
II	January and February	1 0	252
III	March and April	2 6	100
IV	May and June	12 7	224
V	July and August	3 0	157
VI	September and October	32 5	272
VII	November and December	11 5	549

It will be noted from the graph (Fig 1), prepared from Table I, that the curve of 'r'-values—i e , of thyroid-size—follows closely upon that of the rainfall The closeness with which it does so is remarkable and can hardly be regarded as accidental, though further observations are needed to establish the constancy of the association

Apart from any influence which rainfall may have on the goitrogenic potency of cabbage grown in this neighbourhood, the graph (Fig 1) illustrates the extent to which this potency varies at different seasons of the year It is to be noted that it was slight or nil during the months of January to April This effect of season may be illustrated by the results of another experiment Twenty-four rabbits were fed throughout the year—April 1931 to April 1932—on an exclusive diet of fresh, raw cabbage and water These may be referred to as Group A Fifteen survived the experiment, their body-weights remained fairly constant They were killed during the month of April 1932, the mean 'r'-value of their thyroid glands was found to be 260 mg They had received during the months of November and December (1931) the same cabbage as another group (VII), consisting also of 24 animals, which was fed upon it for 56 days only during these months Of the latter 21 survived the experiment, these were killed at the end of December They were found to be the subjects of well-marked goitres, the mean thyroid-size ('r') being 549 mg, or twice as large as in rabbits fed on cabbage the whole year round It is reasonable to suppose that during the months of November and December the size of the gland in Group A was no less than in Group VII,

since the animals in both groups were fed on the same cabbage If this be so it would follow that from January onwards to April a reduction in the size of the gland occurred in Group A—a process of spontaneous cure resulting from the loss, during January to April, of the goitrogenic potency of the cabbage

These preliminary considerations are essential to an understanding of the dietetic conditions prevailing in the experiment with which this paper deals

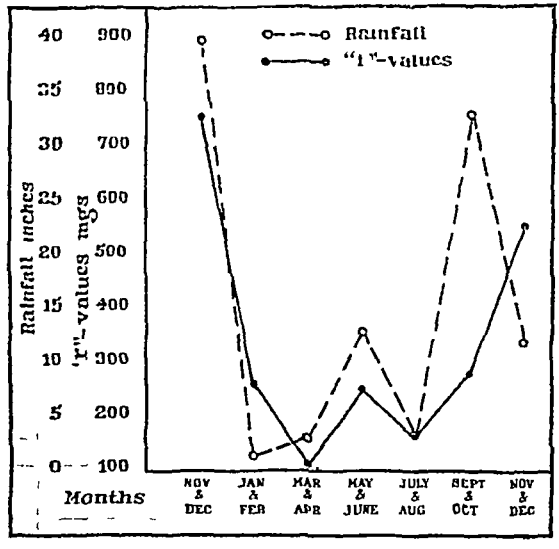

Fig 1 Showing association between rainfall and size ('r'= thyroid weight per kilogram of body weight) of the thyroid gland in rabbits fed on an exclusive diet of fresh raw cabbage

The experiment

This experiment was designed, (*a*) to confirm the results previously recorded (McCarrison and Madhava, 1932) regarding the goitrogenic influence of insanitary condition in albino rats fed on an inferior diet, (*b*) to learn whether or not the goitrogenic agent associated with insanitary condition had any influence on organs of the body other than the thyroid gland, (*c*) to ascertain what effect the 'goitre'—as opposed to the agent causing it—had upon the heart and other organs

For many years past the writer has maintained that a causal relationship exists between insanitary condition and 'goitre', and much evidence—epidemiological, therapeutic and experimental—has, from time to time, been brought forward in support of this contention But he has been well aware that insanitary condition does not always cause goitre, and his problem of late years, has been to learn why

it should be goitrogenic at one time, or in one set of circumstances, and not at another One reason for this has been found to lie in diet a properly constituted diet appears to afford animals (albino rats) complete protection against the goitre so produced In fulfilment of the purposes in view it was necessary, therefore, to devise a diet which, while not in itself goitre-producing or which exerted only a feeble goitrogenic action would admit of the operation of the goitrogenic agent associated with insanitary condition Such a diet—suitable for use with albino rats—is one composed of 60 parts of fresh, raw cabbage, 20 parts of whole wheat flour, 20 parts of *cholam* (a kind of maize) and distilled water, always provided that the experiment in which this diet is used is carried out at a season of the year when the goitrogenic potency of cabbage is low It was necessary, also, to use standardized animals obtained from a perfectly healthy stock in which the size of the thyroid gland at any given body-weight is known to conform to a pre-determined formula (McCarrison and Madhava, 1932) It was necessary, further, that the experiment should be on a scale considerable enough to admit of statistical analysis of the results yielded by it

By these means it was hoped to provide further, and conclusive, evidence of the goitrogenic influence of insanitary condition and to clear the way for a study of its effects on other organs of the body besides the thyroid gland

The possibility that the 'goitre-noxa' may have an injurious effect on the organism has been suggested by several distinguished investigators of 'endemic goitre' De Quervain (1927), in particular, considers that such an assumption is essential to a right understanding of certain clinical manifestations of 'endemic cretinism' But the demonstration of any such effect has not hitherto been possible 'because we are not sufficiently acquainted with the nature of the hypothetical goitre-noxa' (de Quervain, 1927) It is true that research has so far failed to reveal any single, specific agent which is the universal cause of goitre in all parts of the world But it has revealed a number of goitrogenic agents or influences, both of a negative and of a positive kind (McCarrison and Madhava, 1932) Of these influences insanitary condition is one, and although we do not yet know the precise nature of the 'goitre-noxa' associated with insanitary condition yet it is possible to ascertain the effects of this condition on the animal organism and to learn what changes are brought about by it in other organs of the body coincident with those occurring in the thyroid gland In this paper we record the results of a preliminary study of these effects in so far as they are demonstrable by significant changes in the weights of the organs concerned In subsequent papers it is hoped to consider these effects from other angles and to deal with those produced by others of the known goitrogenic agents

Another matter which has seemed to call for elucidation is the supposed association between goitre and cardiac enlargement It has for long been asserted that in experimentally produced rat-goitre such an association exists The writer has,

however, consistently failed to find it Nor can any such association, or the want of it be determined with accuracy unless the rats employed for its ascertainment are taken from a standardized stock in which the variability in size of the heart and of the thyroid gland as well as of other organs, is reduced to the lowest possible minimum by minute attention to nutritional and hygienic conditions of life The present experiment afforded an opportunity to re-examine this question

The experiment was carried out during the period of January to April (*vide* Fig 1), that is to say, during the hot, dry season of low rainfall when the goitrogenic potency of cabbage is at its lowest It lasted for 110 to 120 days The animals used were young albino rats taken from the standardized stock maintained in this Laboratory This stock is an unusually healthy one (McCarrison, 1931) and, in it, goitre is conspicuous by its rarity The body-weights of the selected animals ranged between 36 and 80 grammes a range sufficiently wide for the purposes in view They were fed on *the cabbage-wheat-and-cholam diet* above referred to Its chemical composition was as follows —

Moisture	60 200 per cent
Mineral matter	1 172 ,,
Ether extractives	1 329 ,,
Crude proteins	5 548 ,,
Crude fibre	1 330 ,,
Carbohydrates (by difference)	30 421 ,,
TOTAL	100 000

Insoluble mineral matter	0 198 per cent
Soluble mineral matter	0 974 ,,
Phosphoric acid (P_2O_5)	0 439 ,,
Iron oxide (Fe_2O_3) } Alumina (Al_2O_3) }	0 0134 ,,
Lime (CaO)	0 078 ,,
Magnesia (MgO)	0 054 ,,
Potash (K_2O)	0 235 ,,
Sulphates (SO_3)	0 051 ,,
Soda (Na_2O)	0 136 ,,

This diet is deficient in fats and in suitable protein It does not appear to be lacking in vitamins B_1 and C, but its content of vitamin B_2 is low It is deficient in vitamin D, and, if we are to judge by the causes of death in rats fed upon it (chiefly infection of the lungs), it cannot be regarded as containing an optimum amount of vitamin A Nevertheless, no obvious signs of deficiency either of vitamin A or vitamin D were apparent in the animals within the period of the experiment Its iodine-content was not determined, but it will be evident from the results of

the experiment that neither was this so low as to be a cause of goitre in rats living in sanitary conditions of life nor so high as to prevent goitre in those living in insanitary conditions The urinary excretion of iodine by rats fed upon it averaged 50 γ per litre under the former conditions and 45 γ under the latter

The rats were divided into two groups whose aggregate body-weights were approximately the same The first group—hereinafter referred to as 'sanitary'—consisted of 66 males and 63 females ' This group lived under conditions of scrupulous cleanliness, each animal being confined in a separate, screened cage which was washed at frequent intervals with cresol solution The second group—hereinafter referred to as 'insanitary'—consisted of 63 males and 70 females * This group lived throughout the same period under conditions of gross insanitation These conditions were as follows —

The cages used in a previous experiment of the same kind were utilized in this one, the excreta and decaying food which had accumulated in them from the prior experiment being allowed to remain Each cage was of the dimensions 2 ft by 3 ft by 1 8 ft , its floor being of soft wood Twenty to twenty-five of the rats were placed in each of six such cages, the sexes being separated so as to avoid the effect of sexual activity in causing a physiological increase in size of the thyroid gland (McCarrison and Madhava, 1932) What with the excreta already present in these cages at the commencement of the experiment and those deposited during its course a mound six or more inches thick accumulated in each, into it the animals burrowed The season being hot and dry the mounds of excreta and decaying food were kept moist, by sprinkling them with water from time to time, so as to encourage fermentation which was further enhanced by the presence of yeasts The food of the animals was thrown to them on these mounds, and such remnants as remained uneaten were allowed to augment the mass of decaying organic matter The conditions of insanitation were thus of a very gross kind, more so, indeed, than in any previous experiment of this nature carried out in these Laboratories

At the end of the experiment the rats were killed by drowning and all organs with the exception of the brain, the pituitary body, the pancreas and the lungs were removed and weighed The data thus provided relate, therefore, to body-weights and to the weights of the thyroid, the adrenals, the spleen, the testicles, the thymus, the liver, the kidneys and the heart

Effect of insanitary condition on the body-weight.

This effect is shown in Table II and in Fig 2 From these it will be seen that insanitation had little or no effect on body-weight

* These numbers relate only to the animals that survived the experiment During its course a few died in each group, the chief cause of death was pneumonia, a less common cause was *Bartonella muris* anæmia

TABLE II

Showing the frequency distributions of body-weights of rats living in sanitary and in insanitary conditions (Fig 2)

Range of body weight, g	Number of rats		Range of body weight, g	Number of rats	
	Sanitary	Insanitary		Sanitary	Insanitary
60—69		1	Brought forward	129	132
70—79	1	1	210—219		
80—89		2	220—229		1
90—99	4	3			
100—109	16	7	TOTALS	129	133
110—119	26	20			
120—129	14	20	Mean and standard error	138 10 ± 2 30	143 35 ± 2 67
130—139	4	15	Standard deviation (σ)	25 98	30 63
140—149	12	10			
150—159	18	8	Co efficient of variability	19	21
160—169	16	14			
170—179	16	12			
180—189	2	13	Difference between means	5 25 ± 3 52	
190—199		5			
200—209		1	Significance	t = 1 5, not significant	
Carried over	129	132			

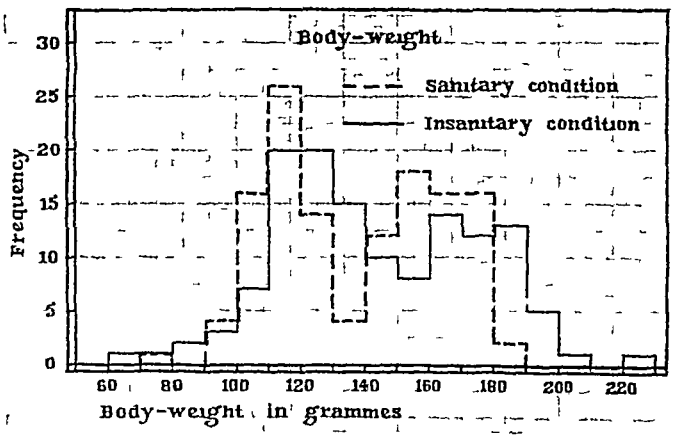

Fig 2 Frequency distributions of body weights of albino rats living in sanitary and in insanitary conditions while being fed on the same diet Note the practical identity of the distributions in the two groups

It is true that the mean body-weight in both males and females was slightly greater in the 'insanitary' group But the excess is not significant and, such as it is, it may have been related as much to the isolation of the one group as to the insanitation of the other In both groups the mean body-weight was approximately 40 grammes less in females than in males The practical identity of the final body-weights in the two groups makes their comparison all the more precise and allows of the use either of the actual organ-weights or of the 'r'-values of the various organs for statistical purposes By using both, as we have done, the results derived from the one serve to control those derived from the other

Effect of insanitary condition on the thyroid gland.

This effect is revealed in Tables III(*a*) and III(*b*) wherein the mean thyroid-weights, the mean thyroid-sizes ('r'), and the frequency distributions of thyroid-weights and thyroid-sizes are set out in parallel columns for the two groups It is shown graphically in Figs 3 to 6

TABLE III(*a*)

Showing mean thyroid-weights and mean thyroid-sizes ('r') in rats living in sanitary and in insanitary conditions (Figs 3 and 4)

Body weight, g	Number of rats		Thyroid weight, mg		Thyroid size, mg	
	Sanitary	Insanitary	Sanitary	Insanitary	Sanitary	Insanitary
60—69		1		12 2		18 8
70—79	1	1	6 7	7 0	8 5	9 2
80—89		2		17 8		10 4
90—99	4	3	8 7	11 1	8 9	11 8
100—109	16	7	7 5	16 6	7 2	15 4
110—119	26	20	7 7	16 6	6 7	14 5
120—129	14	20	8 2	18 4	6 6	14 8
130—139	4	15	7 5	21 3	5 7	16 0
140—149	12	10	8 0	21 8	5 6	15 1
150—159	18	8	8 2	22 7	5 3	14 8
160—169	16	14	8 3	22 6	5 1	13 7
170—179	16	12	9 8	29 2	5 6	16 5
180—189	2	13	10 2	32 7	5 6	17 8
190—199		5		36 7		18 7
200—209		1		37 8		18 4
210—219	.	.			.	
220—229	..	1		35 2		15 4

TABLE III(*b*)

Showing frequency distributions of thyroid-weights and thyroid-sizes ('1') in rats living in sanitary and in insanitary conditions (Figs 5 and 6)

Range of thyroid weight, mg	Number of rats		Range of thyroid size, mg	Number of rats	
	Sanitary	Insanitary		Sanitary	Insanitary
3 0—5 9	6		3 0— 3 9	1	
6 0—8 9	82	3	4 0— 4 9	23	
9 0—11 9	39	8	5 0— 5 9	50	
12 0—14 9	2	11	6 0— 6 9	23	1
15 0—17 9		20	7 0— 7 9	17	1
18 0—20 9		26	8 0— 8 9	7	1
21 0—23 9		21	9 0— 9 9	6	2
24 0—26 9		14	10 0—10 9	1	9
27 0—29 9		9	11 0—11 9	1	9
30 0—32 9		6	12 0—12 9		13
33 0—35 9		5	13 0—13 9		16
36 0—38 9		3	14 0—14 9		17
39 0—41 9		3	15 0—15 9		19
42 0—44 9		2	16 0—16 9		10
45 0—47 9			17 0—17 9		8
48 0—50 9		1	18 0—18 9		8
51 0—53 9		1	19 0—19 9		4
			20 0—20 9		4
			21 0—21 9		2
			22 0—22 9		4
			23 0—23 9		3
			24 0—24 9		
			25 0—25 9		
			26 0—26 9		1
			27 0—27 9		1
TOTALS	129	133		129	133
Mean and standard error	8 39 ± 0 14	22 27 ± 0 73		6 69 ± 0 12	15 71 ± 0 32
Standard deviation (σ)	1 57	8 44		1 37	3 71
Coefficient of variability	19	38		21	24
Difference between means	13 97 ± 0 75			9 10 ± 0 34	
Significance	t = 18 6, significant			t = 26 8, significant	

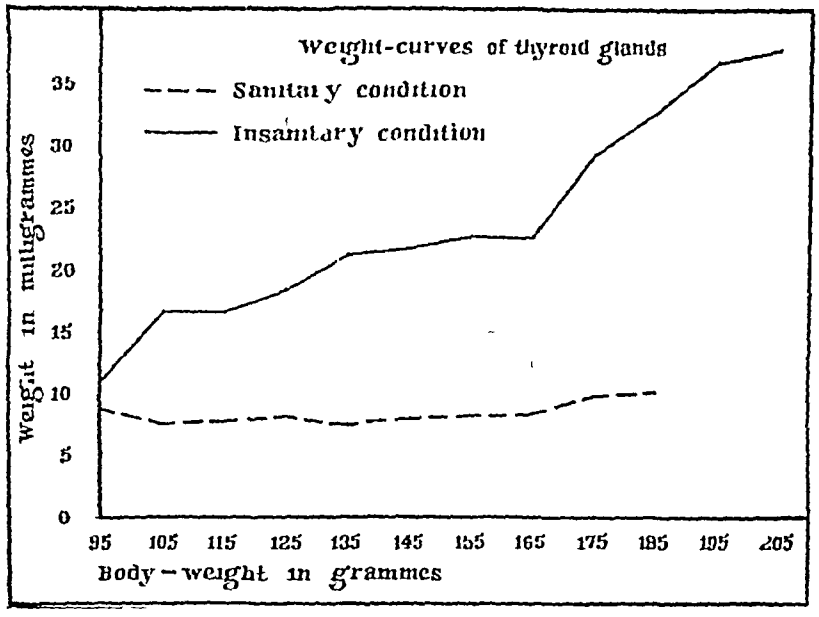

Fig 3 Weights of the thyroid gland at different body weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the greater and progressively increasing weight of the gland in the insanitary group

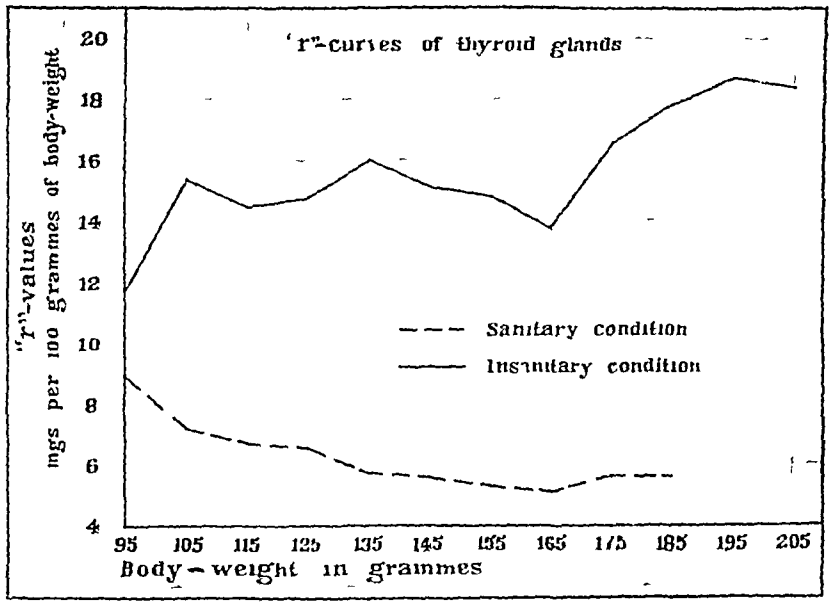

Fig 4 Size ('r') of the thyroid gland at different body weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the progressively increasing difference in size of the gland in the two groups with increasing body-weight

It will be noted, in the first place, that the experimental diet was not in itself goitre-producing The mean 'r'-value of the thyroid gland was 6 64 ± 0 12 in the 'sanitary' group as compared with 9 76 ± 0 11 in well-fed stock rats, a difference which is significant and indicates that, so far from the experimental diet being goitrogenic, it caused the thyroid gland to be smaller than normal Its size was in conformity with that of the gland (7 04 ± 0 11) in rats fed on purely vegetarian, but otherwise well-balanced, diets (McCarrison and Madhava, 1932) It will be observed also that the action of the experimental diet on the gland was an uniform one, the co-efficient of variability of 'r' being only 20 per cent

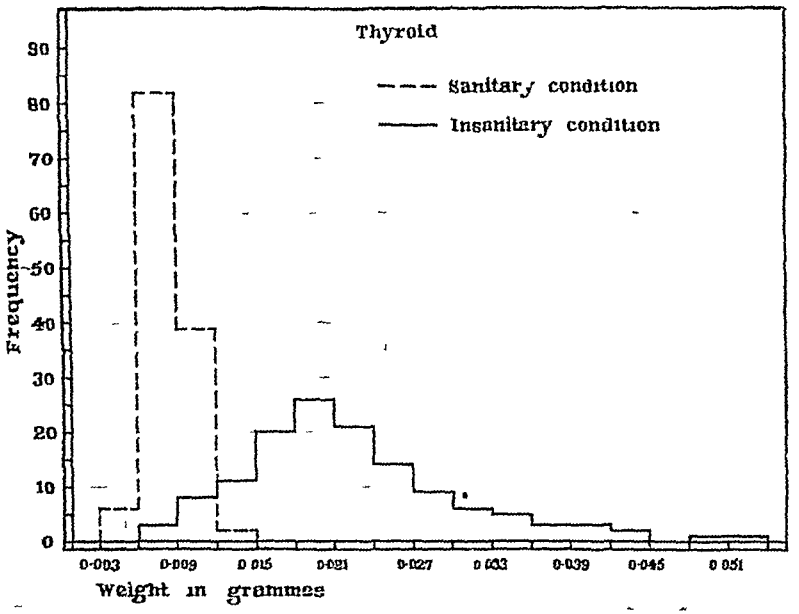

Fig 5 Frequency distributions of thyroid weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note narrow limits and low range of the distribution in the sanitary group, the wide limits and high range in the insanitary group, the positive skewness and the later occurrence of the modal point in the latter

In contrast to the non-goitrogenic action of the diet in this experiment its goitrogenic action in a previous one (McCarrison and Madhava, 1932) is of interest The previous experiment lasted much longer (274 days) and extended from January to October thus including the period of summer rains The mean 'r'-value of the thyroid gland was 10 38 ± 0 33 as compared with 6 64 ± 0 12 in the present experiment The difference between these two values is significant, it may be attributable in some part to the longer duration of the previous experiment but

the greater goitre-producing potency of cabbage during the period from May to October is probably responsible for it

In contrast to the small size of the thyroid gland in the ' sanitary ' group its uniformly large size in the ' insanitary ' group is very striking It will be noted, both from the actual thyroid-weights and from their equivalent ' r '-values, that this increase occurs at every range of body-weight The mean ' r '-value is 15 74 ± 0 32 as compared with 6 64 ± 0 12 in the ' sanitary ' group , the difference between these means is very significant Here again the co-efficient of variability in thyroid-size

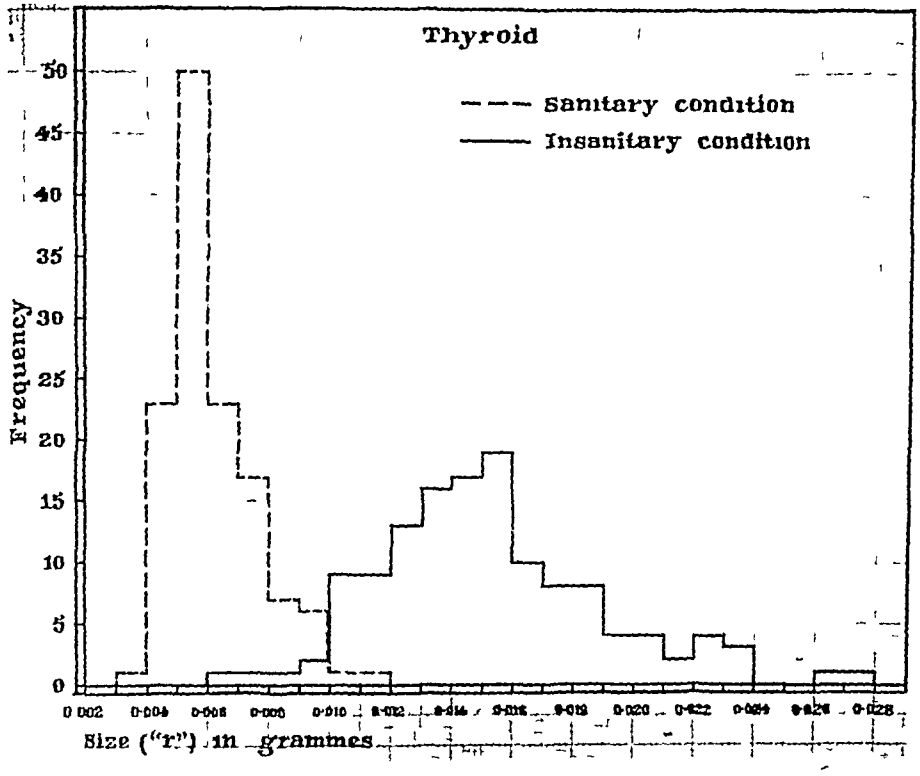

Fig 6 Frequency distributions of thyroid sizes (' r ') in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the same features as in Fig 5

(' r ') is low—24 per cent—indicating the uniformity of action of the ' goitre-noxa ' associated with insanitary condition This action is well illustrated in Figs 5 and 6 showing the frequency distributions of thyroid-weights and of thyroid-sizes (' r ') It will be noted that the diagrams not only reveal a high degree of positive skewness in the ' insanitary ' group—the larger weights and sizes occurring in the right hand side of the diagrams—but the modal point occurs much later in this group These results demonstrate conclusively that under the dietetic conditions of this experiment insanitary condition exercised a markedly goitrogenic

action Confirmation of previous results is thus afforded (McCarrison and Madhava, 1932)

It is of interest to contrast the present results with those previously reached As stated above the goitre-producing potency of the cabbage used on the previous occasion was, in general, greater and the duration of the experiment longer than in the present one, yet the mean 'r'-value of the thyroid gland of rats living under insanitary conditions was less 12 21 ± 0 44, as compared with 15 74 ± 0 32 in the present experiment The difference between these values is significant It may, therefore, be concluded that the conditions of insanitation prevailing in the present experiment were more potent to cause goitre than those prevailing in the previous one It would seem that the kind or degree of insanitation is a matter of importance in relation to the genesis of goitre in rats fed on the inferior diet

Fig 4 brings into prominence another point of interest It will be noted from it that while the 'r'-curve of rats living in sanitary conditions falls, with increasing body-weight, in a manner similar to that of the normal 'r'-curve of the thyroid gland (McCarrison and Madhava, 1932), that of rats living in insanitary conditions rises continuously, departing more and more from the normal course The action of the 'goitre-noxa' associated with insanitary condition is not only to cause thyroid enlargement but to cause the enlargement to increase in size with increasing body-weight and age

[*To be concluded in the January 1933 number of the* Journal —Ed]

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MINERAL METABOLISM IN RABBITS FED ON A CABBAGE DIET

BY

S RANGANATHAN, M SC,

Nutritional Research Laboratories, I R F A, Pasteur Institute, Coonoor, S India

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THE studies in mineral metabolism dealt with in this paper were undertaken in connexion with the investigations on goitre now proceeding in these Laboratories (McCarrison, 1931, McCarrison and Madhava, 1932) Their object was, first, to investigate the metabolism of calcium, magnesium and phosphorus in rabbits fed on an exclusive diet of fresh, raw cabbage and, secondly, to observe the changes, if any, brought about by the addition of lime or sodium phosphate to this diet Baumann, Kurland and Metzer (1931) have reported that the hyperplastic goitres produced in rabbits by an exclusive diet of cabbage are associated with a retention of calcium, magnesium and phosphorus in the body They assume that the calcium retention is directly connected with the development of these goitres, having found that the administration of iodine reduces the positive balance to zero while the animals are still being fed on the cabbage diet If positive calcium-balance be the prime factor in goitre-production in rabbits fed on a cabbage diet and if iodine be able to prevent it by reducing this positive balance to zero, then it may be supposed that any substance which is physiologically antagonistic to calcium would be as likely to prevent this type of goitre as iodine, further, an excess of calcium in the diet should aggravate it

Experimental.

Twelve rabbits, six males and six females, each weighing approximately 1,600 g were used They were confined in individual metabolism cages and divided into four groups of three animals They were fed as follows —

Group I received the 'stock' diet consisting of cabbage, grass, carrots, bran and sprouted gram Of these constituents, each animal received

daily 500 g of fresh raw cabbage, 10 g of grass, 10 g of bran, 50 g of carrots and 50 g of sprouted gram

Group II received fresh, raw cabbage only 600 g per rabbit per day

Group III received cabbage, as in group II, together with 0 1785 g. of slaked lime per rabbit per day

Group IV received cabbage as in group II, together with 0 29 g of sodium phosphate (Na_2HPO_4, $12H_2O$) per rabbit per day

The animals were provided with distilled water *ad libitum* The duration of the experiment was 54 days

The weighed amounts of the sodium phosphate or of the slaked lime were made into a thick paste with a little distilled water, smeared over bits of tender cabbage, and given first thing in the morning to the hungry animals When these were eaten, the cabbage ration was then given The animals were fed at 11 A M daily and the food left unconsumed was picked out the next morning and weighed The calcium, magnesium and phosphorus content of the various diets were estimated The urines were collected daily and analyses made on representative samples of them collected during each period of four days Urinary deposits were brought into solution by acidifying the urine with about 10 c c of concentrated hydrochloric acid Great precautions were not taken to prevent fermentation of the urine and the fæces, as the investigation was limited to mineral metabolism alone The fæces were collected daily and at the end of each period of four days they were dried at 100°C for over six hours, when completely dry, they were weighed, powdered and representative samples used for analysis Their contents of calcium magnesium and phosphorus were then determined By these means it was possible to arrive at a fairly accurate estimate of the intake of calcium, magnesium and phosphorus, and of the respective amounts of them that were excreted through the urinary and intestinal tracts, and therefrom to estimate the amounts retained in the system The results of the analyses, extending over six periods, are recorded in Tables I(*a*), (*b*) and (*c*)

It will be seen from Tables I(*a*), (*b*) and (*c*) that approximately the same amount of calcium was excreted in all the groups The amounts retained were also much the same in groups I, II and III, viz, about 36 per cent of the intake Group III, which received lime in addition to the cabbage, retained more, the retention being about 46 per cent of the intake The corresponding results of Baumann and his co-workers calculated for a like period would be as follows The two rabbits, Nos 641 and 657, maintained by them on an exclusive diet of cabbage, retained on an average 0 718 g and 0 451 g respectively of CaO in four days, while the control rabbit fed on 'stock' diet retained 0 410 g of CaO under similar conditions

TABLE I(a)

Showing the metabolism of calcium

	CALCIUM AS CaO					
	Intake, mg	Output			Balance or retention	
		Urine, mg	Faeces, mg	Total, mg	Mg	Percentage of intake
			Group I Controls on 'stock' diet			
	1,670 3	670 0	294 0	964 0	706 3	42 3
	1,690 7	471 0	462 0	933 0	757 7	44 8
	1,634 9	162 0	414 0	876 0	758 9	46 4
	1,708 3	450 0	959 0	1,409 0	299 1	17 4
	1,708 1	630 0	635 0	1,265 0	443 1	26 0
	1,659 5	452 0	758 0	1,210 0	449 5	27 1
Mean	1,678 6	522 5	587 0	1,109 5	569 1	34 0
			Group II Raw cabbage only			
	1,540 0	552 0	327 0	879 0	661 0	43 0
	1,274 0	494 0	352 0	846 0	428 0	33 6
	1,394 0	250 0	718 0	968 0	426 0	30 7
	1,462 0	425 0	530 0	955 0	507 0	34 6
	1,350 0	395 0	207 0	602 0	748 0	55 3
	1,496 0	408 0	811 0	1,219 0	277 0	18 6
Mean	1,419 3	420 7	490 8	911 5	507 9	36 0
			Group III Raw cabbage + lime			
	2,039 2	533 0	246 0	779 0	1,260 2	62 0
	1,972 4	793 0	304 0	1,097 0	875 4	44 4
	2,038 4	313 0	345 0	658 0	1,380 4	67 9
	2,038 4	699 0	690 0	1,389 0	649 4	31 8
	2,038 0	603 0	746 0	1,349 0	689 0	33 8
	2,038 4	330 0	930 0	1,260 0	778 4	38 1
Mean	2,027 5	545 2	543 5	1,088 7	938 8	46 3
			Group IV Raw cabbage + sodium phosphate			
	1,564 2	127 0	313 0	440 0	1,124 2	71 8
	1,468 2	380 0	466 0	846 0	622 2	42 4
	1,570 0	368 0	597 0	965 0	605 0	38 5
	1,570 2	539 0	641 0	1,180 0	390 2	24 9
	1,570 2	600 0	610 0	1,210 0	360 2	22 9
	1,570 2	557 0	550 0	1,107 0	463 2	29 5
Mean	1,552 2	428 5	529 5	958 0	594 2	38 3

TABLE I(*b*)

Showing the metabolism of phosphorus

	PHOSPHATES—P_2O_5					
	Intake, mg	OUTPUT			BALANCE OR RETENTION	
		Urine, mg	Fæces, mg	Total, mg	Mg	Percentage of intake
			GROUP I Controls on 'stock' diet			
	2,292 0	408 0	343 0	751 0	1,541 0	67 3
	2,373 0	519 0	909 0	1,428 0	945 0	39 9
	2,406 0	382 0	682 0	1,064 0	1,342 0	56 0
	2,428 0	147 0	1,040 0	1,187 0	1,241 0	51 1
	2,432 0	452 0	1 036 0	1 488 0	944 0	39 0
	2,344 0	253 0	952 0	1,205 0	1 139 0	48 6
Mean	2,379 2	360 2	827 0	1,187 2	1,192 0	50 3
			GROUP II Raw cabbage only			
	1,840 0	138 0	217 0	355 0	1,485 0	80 7
	1,524 0	496 0	152 0	648 0	876 0	57 4
	1,742 0	119 0	550 0	669 0	1,073 0	61 4
	1,756 0	79 0	490 0	569 0	1,187 0	67 7
	1,616 0	177 0	276 0	453 0	1,163 0	72 0
	1,796 0	136 0	770 0	906 0	890 0	49 5
Mean	1,712 3	190 8	409 2	600 0	1,112 3	64 8
			GROUP III Raw cabbage + lime			
	1,865 2	160 0	188 0	348 0	1,517 2	81 3
	1,767 2	280 0	280 0	560 0	1,207 2	68 3
	1,849 2	144 0	349 0	493 0	1,356 2	73 1
	1,849 2	212 0	892 0	1,104 0	745 2	40 4
	1,845 2	90 0	617 0	707 0	1,138 2	61 7
	1,849 2	115 0	767 0	882 0	967 2	52 3
Mean	1,837 5	166 8	515 5	682 3	1,155 2	62 9
			GROUP IV Raw cabbage + sodium phosphate			
	2,106 0	112 0	151 0	263 0	1,843 0	87 5
	1,988 0	339 0	553 0	892 0	1,096 0	54 9
	2,108 0	159 0	570 0	729 0	1,379 0	70 0
	2,112 0	138 0	445 0	583 0	1,529 0	72 1
	2,112 0	240 0	620 0	860 0	1,252 0	59 2
	2,112 0	227 0	560 0	787 0	1,325 0	63 0
Mean	2,089 7	202 5	483 2	685 7	1 404 0	67 8

TABLE I(c)

Showing the metabolism of magnesium

MAGNESIUM AS MgO

	Intake, mg	Output			Balance or retention	
		Urine mg	Fæces, mg	Total, mg	Mg	Percentage of intake
			Group I Controls on 'stock' diet			
	1,251 2	252 0	144 0	396 0	855 2	68 5
	1,298 1	375 0	426 0	801 0	497 1	38 4
	1 330 6	334 0	399 0	733 0	597 6	44 9
	1 330 6	432 0	648 0	1,080 0	250 6	18 9
	1 333 6	448 0	314 0	762 0	571 6	43 0
	1,285 6	417 0	345 0	788 0	495 6	38 5
Mean	1 304 6	380 7	379 3	760 0	544 6	42 0
			Group II Raw cabbage only			
	1,000 0	159 0	82 0	241 0	759 0	75 9
	828 0	236 0	68 0	304 0	524 0	63 3
	905 0	195 0	317 0	512 0	393 0	43 5
	950 0	440 0	296 0	736 0	214 0	22 5
	875 0	374 0	102 0	476 0	399 0	45 5
	970 0	374 0	294 0	668 0	302 0	31 2
Mean	921 3	296 3	193 2	489 5	431 8	47 0
			Group III Raw cabbage + lime			
	1,012 0	140 0	66 0	206 0	806 0	79 6
	965 6	112 0	84 0	196 0	769 6	79 7
	1,007 6	290 0	193 0	483 0	524 6	52 0
	1,007 6	449 0	311 0	760 0	247 6	24 5
	1,005 6	473 0	424 0	897 0	108 6	10 8
	1 007 6	403 0	287 0	690 0	317 6	31 5
Mean	1,001 0	311 2	227 5	538 7	462 3	46 3
			Group IV Raw cabbage + sodium phosphate			
	999 0	57 0	61 0	118 0	881 0	88 1
	940 0	257 0	172 0	429 0	511 0	54 4
	1,003 1	318 0	285 0	603 0	400 1	39 9
	1,001 1	387 0	253 0	640 0	364 1	36 3
	1,004 1	403 0	391 0	794 0	210 1	21 0
	1,004 1	354 0	324 0	678 0	326 1	32 5
Mean	992 4	296 0	247 7	543 7	448 7	45 4

There was a greater excretion of phosphates (1,187 2 mg) in group I than in other groups (600 0 mg in group II, 682 3 mg in group III and 685 7 mg in group IV), while the amounts retained were roughly the same (about 1,153 2 mg) in all the groups except in group IV (1 404 0 mg) which received phosphate in addition to the cabbage The corresponding values of Baumann and his colleagues for their two rabbits are 0 844 g and 0 714 g respectively of P_2O_5 in four days Their figures, calculated as a percentage of the intake, work out at 54 9 and 59 2 per cent respectively, while in the present investigation the average retention of P_2O_5 during the six periods was 64 8 per cent of the intake The figure for rabbits fed on ' stock ' diet was not very different from that found in those maintained on an exclusive diet of cabbage, Baumann and his co-workers however report negative values for phosphate retention in rabbits fed on the ' stock ' diet used by them A ' stock ' diet which occasions a regular depletion of phosphates from the system can hardly be regarded as satisfactory The same comment applies to their observations regarding the retention of magnesium their ' stock ' diet leads to a systematic depletion of this element The ' stock ' diet employed in this Laboratory has always been found to give positive values for magnesium retention

Serum-calcium values.

Dupré and Semeonoff (1931) report wide variations in the serum-calcium values of rabbits, the figures ranging from 7 6 mg to 22 0 mg per 100 c c ' The most common percentage, however, was between 9 5 and 18 5 ' Random variations in the level of serum-calcium of two rabbits, maintained by them on a ' stock ' diet of cabbage, bran and oats, were from 10 6 to 16 6 mg and 9 4 to 15 8 mg respectively With a view to ascertaining the serum-calcium values in the rabbits used in the present experiment, the animals were bled twice, once during the middle of the experiment and again at its close Individual calcium-estimations of the blood sera of the twelve animals were then made by the Clark-Collip's modification of the Kramer and Tisdall's method The results of these estimations are given in Table II

It is to be seen from this table that the serum-calcium values of rabbits fed on a ' stock ' diet of cabbage, bran, sprouted gram, fresh carrots and green grass, do not differ so widely as those in Dupré and Semeonoff's experiment, the figures ranging only from 14 2 to 16 2 mg , with an average of 15 0 mg per 100 c c It may be mentioned here that the bleeding was done in the morning before the animals had fed In the same paper these observers also report that ' cabbage fed to rabbits causes a marked rise in the serum-calcium, and a simultaneous fall in the inorganic phosphorus concentration ' They observe that this rise in serum-calcium is only temporary, taking place immediately after the intake of food, a normal condition prevailing thereafter They do not say that prolonged feeding on cabbage permanently raises the serum-calcium value Table II shows that the serum-calcium

TABLE II

Showing the values of calcium in blood serum

Group and diet	Rabbit number	Milligrams per 100 c c			
		First observation		Second observation	
Group I — 'Stock' diet	264	14 5	Mean = 15 0	14 5	Mean = 15 0
	265	15 8		16 2	
	266	14 6		14 2	
Group II — Raw cabbage only	267	15 0	Mean = 15 1	13 6	Mean = 15 3
	268	14 6		17 6	
	269	15 8		14 6	
Group III — Raw cabbage + lime	270	15 2	Mean = 15 7	15 6	Mean = 14 4
	271	15 2		13 1	
	272	17 2		14 4	
Group IV — Raw cabbage + sodium phosphate	273	15 6	Mean = 15 2	*	
	274	13 8		*	
	275	16 2		12 8	

* These animals died immediately after the first bleeding

of rabbits fed for nearly two months on cabbage alone ranged from only 13 6 to 17 6 with a mean of 15 3 mg per 100 c c , the average value thus being normal No significant change was observed when the cabbage was supplemented with either lime or sodium phosphate

Post-mortem findings

At the close of the experiment the animals were post-mortemed in the Pathological Department and the thyroid glands carefully dissected out They were at once weighed correct to a tenth of a milligram The original and final body-weights, the thyroid-weights and the 'r'-values of the thyroid—that is, the weight of the thyroid per kilogram of body-weight (McCarrison and Madhava, 1932)—are given in Table III The statistical analysis of these figures is given in Table IV

TABLE III

Showing the original and final body-weights and thyroid-size ('r')

Group and diet	Rabbit number	Original body weight in g	Final body weight in g	Thyroid weight in mg	'r' value in mg
Group I —					
'Stock' diet	264	1,605	2,005	277 4	138 4
	265	1,595	2,030	241 8	119 1
	266	1,650	1,865	220 6	118 3
Group II —					
Raw cabbage only	267	1,430	1,625	155 6	95 8
	268	1,545	1 565	120 6	77 1
	269	1 750	1 855	315 4	170 0
Group III —					
Raw cabbage + lime	270	1,390	1,665	256 4	154 0
	271	1,540	1 580	237 2	150 1
	272	1,525	1,700	225 8	132 8
Group IV —					
Raw cabbage + sodium phosphate	273	1,300	1,275	373 6	293 0*
	274	1,990	1,825	126 8	69 5*
	275	1,515	1,610	152 0	94 4

* These two animals died during the course of the experiment

A perusal of Tables III and IV reveals the superiority of the 'stock' diet This is evidenced by the considerable increase in body-weight (21 6 per cent in 54 days) and by the uniformity of growth as indicated by the small coefficient of variation (3 7) in final body-weight Its action on the thyroid gland was a very uniform one, the coefficient of variability of thyroid-weight being only 9 5 and of the thyroid-size only 7 4 The cabbage diet to which lime was added (group III) yielded results which approximated in some respects to those yielded by the 'stock' diet, but the mean final body-weight was lower while the thyroid-size was higher than in the animals fed on the 'stock' diet In this group two out of three animals were goitrous, this high incidence of goitre, taken in conjunction with the low

and 272 (group III) may be considered to have had slight, though insignificant, enlargement of the thyroid gland since the '*t*'-values were greater than the mean in controls by about once the standard deviation The remaining six animals had no enlargements of the thyroid gland

Thyroid-size and excretion and retention of minerals.

The influence of the diets on the metabolism of calcium, magnesium and phosphorus is shown in Tables I(*a*), (*b*) and (*c*) It remains to be seen whether or not there is any association between thyroid enlargement and the excretion and retention of these minerals The figures, calculated for individual animals and arranged in the ascending order of thyroid-size, are shown in Table V

TABLE V

Contrasting the size ('r') of the thyroid gland with the amounts of calcium, magnesium and phosphorus retained

Serial numbers	Rabbit number	Thyroid size in ascending order in mg	Total excretion in mg			Retention in mg		
			CaO	MgO	P_2O_5	CaO	MgO	P_2O_5
1	274	69 5	846 0	429 0	892 0	622 2	511 0	1,096 0
2	268	77 1	724 0	390 0	550 5	588 0	461 5	1,019 5
3	275	94 4	1,115 5	678 8	734 8	454 7	325 1	1,371
4	267	95 8	917 0	488 5	462 0	584 0	486 5	1,3
5	266	118 3	1,043 0	760 5	1,134		546 6	1,
6	265	119 1	1,099 0	781 5	1,458		534 4	
7	272	132 8	959 0	586 5	68		421 1	
8	264	138 4	1,186 5	738 0	9(		552 9	
9	271	150 1	1,223 0	546 5	6		439 1	
10	270	154 0	1,084 0	483 0	7		526 8	
11	269	170 0	1,093 5	590 0	7		347 5	
12	273	293 0	440 0	118 0	2		881 0	

The above figures represent an average excretion or re for

It will be seen from Table V that rabbit No 273 is in marked contrast to the rest, for, while in the first eleven animals there is no correlation between the size of the thyroid gland and the excretion and retention of calcium, magnesium and phosphorus, yet in the twelfth animal, whose thyroid gland is markedly larger than any of the others, there is a great retention of these elements So far as these observations go, it may be said that when the size (' r ') of the thyroid gland does not exceed 170 mg per kilo of body-weight, there is no association between it and the excretion and retention of calcium, magnesium and phosphorus When the thyroid-size reached 293 mg, a great retention of these elements did occur As this result was observed in only one animal, it is necessary to repeat the experiments during the autumn rains when the goitrogenic potency of cabbage grown in this locality is very high (McCarrison and Madhava, 1933) Then only will it be possible to determine whether retention of these elements is associated with the development of the goitres

The ' stock ' diet of alfalfa hay and oats, employed by Baumann and his colleagues, does not seem to be satisfactory, as their metabolic studies disclose a negative balance of phosphorus and magnesium The ' stock ' diet employed in this Laboratory shows a good retention of these two elements, it is not, however, an optimum diet so far as phosphorus and calcium retention is concerned, since it gives rise to a greater retention of the former than of the latter If the calcium and phosphorus were to be retained mostly in the form of calcium phosphate, they should be stored in the approximate proportion of 2 of Ca to 1 of P Baumann and his co-workers report a total retention of 3 460 g of Ca and 2 487 g of P in their rabbit No 641 and 2 173 g of Ca and 2 103 g of P in their rabbit No 657 Evidently, more phosphorus than is theoretically needed to form calcium phosphate was retained The results of this Laboratory show a similar finding in all the four groups This would seem to be an inherent defect of diets of which cabbage forms a major part The wide disparity in the amounts of calcium and phosphorus retained is minimized when lime is added to the cabbage-diet

Summary and conclusions

The metabolism of calcium, magnesium and phosphorus was studied in rabbits fed on four different diets, all of which contained cabbage in high proportion The finding of Baumann and his co-workers that an association exists between calcium retention and the goitre developing in rabbits fed on cabbage diets has not been substantiated when the goitres so produced are of a size ranging between 150 and 170 mg per kilo of body-weight It has, however, been substantiated—though only in one animal—when the thyroid-size reached 293 0 mg per kilogram of body-weight of the animal

The cabbage used in this experiment was of very low goitrogenic potency The addition of lime to the cabbage increased the goitre-producing action of the diet

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CHEMICAL EXAMINATION OF SOME INDIAN MEDICINAL PLANTS *TINOSPORA CORDIFOLIA*, *SOLANUM XANTHOCARPUM* AND *FUMARIA OFFICINALIS*

BY

GAJANAN PURUSHOTTAM PENDSE,

AND

SIKHIBHUSHAN DUTT

(*From the Chemical Laboratory, Allahabad University, Allahabad*)

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Tinospora cordifolia

Tinospora cordifolia commonly known as 'Gulancha' in Bengali and 'Gurach' in Hindustani is a plant of the natural order of Menispermaceæ It is a well-known medicinal plant of long use in Hindu medicine It is a glabrous, succulent, climbing shrub, often reaching a great height and sending down long thread-like aerial roots The plant seems to be particularly fond of climbing up the horny body of large neem trees in the United Provinces The bark is gray or creamy white, deeply cleft with spiral and longitudinal clefts, the space between the clefts usually dotted with large rosette-like lenticels The wood is white, soft and porous and the freshly cut surface soon assumes a yellow tint on exposure to air The branches bear smooth heart-shaped leaves and bunches of red berries The sap is light yellow in colour and viscous, having a peculiar slimy odour and a nauseating bitter taste

As regards its medicinal properties the Hakims consider it to be cold and sedative The fresh plant is said to be more efficacious than the dry It is taken with milk in rheumatism, acidity of the urine and dyspepsia The dry stem can be seen in every drug shop and from it is prepared a kind of starch known in Hindustani as 'Giloe-ka-sat' and in some parts of India as 'Pilo' It is prepared by powdering the stem and washing out the starch with water, the latter generally always retaining

a little of the bitterness of the drug *Tinospora cordifolia* attracted the attention of European medical men in India, and has been favourably spoken of by them as a tonic, antiperiodic and diuretic It is now official in the Pharmacopœia of India and has been introduced in Europe as a specific medicine The medicinal properties of the plant have been ascribed by various authors as due to the presence of *berberine*, without any isolation or identification of the alkaloid It is stated to be an excellent remedial agent in chronic diarrhœa and some forms of chronic dysentery It was prescribed by ancient Hindu physicians in gonorrhœa with advantage It is also regarded by the natives in certain parts of India as an antidote for the bites of poisonous snakes and insects

The stem has been examined by Fluckiger (1884) by boiling it with alcohol and a little hydrate of calcium, evaporating off the alcohol and extracting the residue with chloroform The chloroform extract responded to the tests for an alkaloid and was yellow in colour from which, probably, the author concluded that the plant contained *berberine* The alcoholic extract after it had been exhausted by chloroform as stated above was dissolved in boiling water and precipitated by tannic acid The precipitate thus obtained was mixed with moist lead carbonate, dried and exhausted with alcohol which, on evaporation, yielded the bitter principle By boiling this bitter principle with dilute sulphuric acid sugar was produced and the substance lost its bitterness Neither the original bitter principle nor the product derived from hydrolysis could be crystallized or obtained in sufficiently purified form

The above represents the work that has been done on the plant up to this time On account of the importance of the drug the present systematic analysis was undertaken in order to have a clearer insight into the nature of the active principle of the plant

The presence of an alkaloid in the plant was definitely ascertained by taking the alcoholic extract and treating it with acidulated water and testing the solution with the usual alkaloidal reagents, such as phosphotungstic acid, phosphomolybdic acid, Meyer's, Dennig's, Wagner's, Froede's and Dragendorf's reagents, gold and platinic chlorides, etc All these, however, gave colour reactions or precipitates which were very different from those given by *berberine* Extractions were then made of the powdered and dried plant by means of various solvents such as water, alcohol, petroleum ether, ether and chloroform and the solvents evaporated, but nothing very interesting could be obtained except chlorophyll, sugars and some resins and waxes Extraction by cold one per cent hydrochloric acid for two days and subsequent treatment with sodium carbonate yielded a precipitate which was found to be purely inorganic in character The ' lime method ' of extraction of the alkaloid from the plant with large quantities of the material was tried, but no alkaloid could be isolated As regards the bitter principle in the plant, various attempts were made to isolate it in a pure form, but all ended in failure At best only sticky solids

could be obtained possessing an intensely bitter taste, which systematically baffled all attempts at purification or crystallization and did not dry up completely even when kept in a vacuum desiccator for months

Experimental

The dried and powdered stems of the plant were extracted in 500 g lots by the following solvents individually (and not one after another) in a large Soxhlet's extractor, and the solvent evaporated water, alcohol ether, petroleum ether and chloroform The results are shown below —

Water extract (18 per cent) Dark brown powder with a sugary odour and a definite bitter taste Reduces Fehling's solution quickly on warming and gives faint colour reactions with alkaloid reagents

Alcoholic extract (6 8 per cent) Dark brown viscous substance possessing an offensive odour and an exceedingly bitter taste More definite colour reaction with alkaloid reagents Fehling's solution slightly reduced on warming

Ether extract (1 5 per cent) Dark greenish yellow sticky mass consisting mainly of fats and chlorophyll No alkaloid reaction and no bitter taste

Petroleum ether extract (1 2 per cent) Dark greenish yellow sticky substance with properties similar to the above

Chloroform extract (2 9 per cent) Greenish yellow waxy solid without bitter taste and giving only faint alkaloid reaction Fehling's solution not reduced

Fifty grammes of the aqueous extract were dissolved in hot water and the solution treated with lead acetate The precipitated brownish white lead lake was filtered off and the filtrate treated with hydrogen sulphide in order to remove the excess of lead The filtered liquid on evaporation to dryness yielded a brown sticky solid (0 34 g) which could not be crystallized It gave all the usual reaction of an alkaloid It still contained a large proportion of sugars

Seventy grammes of the aqueous extract were refluxed with 500 c c of chloroform for ten hours and after filtration the solvent was distilled off A greenish yellow waxy solid (4 g) was left behind which was found to be partially soluble in hot alcohol and was therefore separated into two fractions by means of this solvent

Chloroform soluble solid Colourless waxy substance crystallizing from large quantities of methyl alcohol in glistening leaflets It shrinks at 159°C and melts at 173°C to 174°C It contains no nitrogen and does not reduce Fehling's solution under any condition (Found C=54 01 , H=7 02 , $C_9H_{14}O_5$ requires C=53 45 , H=6 9 per cent) The quantity obtained was insufficient for any further examination

Alcohol soluble solid Colourless cylindrical prisms from absolute alcohol melting at 76°C to 77°C It does not contain any nitrogen and is non-alkaloidal in character Under ordinary conditions it does not reduce Fehling's solution, but does so readily on hydrolysis with dilute hydrochloric acid The substance

Enzymes The fresh plant on examination was found to contain about 0 08 per cent of an oxidizing enzyme (quinhydrone reaction), but this was entirely absent in the dried plant

Fumaria officinalis.

Fumaria officinalis (Hindi—'Shâhetarâh' Bengali—'Pipâprâ'), of the natural order of Fumariaceæ, is a herb very common in India It generally grows near marshy lands and in the rainy season, throughout India It is a low green shrub with minute oblong leaves The dry plant is generally much broken up Mixed with it are many globular iridescent capsules, the size of a large pin's head and umbilicate at the top The seeds are dark brown, crested, with hardly any odour The taste of the plant is bitter, slightly acrid and astringent Several species of the family have long been used medicinally on account of their diuretic and alterative properties It is known to remove hepatic obstructions and is an aperient and expellant of humors but more specially of atrabiles It is also a laxative and is beneficial in dyspepsia depending upon the torpidity of the intestines and in scrofulous skin affections

As regards its chemical examination, it is supposed to contain fumaric acid and a base known as 'fumarine', observed first by Peschier and more fully described by Hannon [*Jour Chem Med* (3), **8**, p 305] The plant contains 5 to 6 per cent of the base to which its physiological activity is supposed to be due The base is separated from the salts contained in the plant by the action of caustic alkalis or their carbonates, in the form of curdy precipitates which may be obtained crystalline by spontaneous evaporation of their hot alcoholic solutions

The above represents the work that has been done on the plant The present authors were tempted to put the entire plant under systematic chemical examination on account of the large amount of crystalline organic base (5 to 6 per cent) supposed to be contained in the plant [illegible] none of the extravagant claims in this direction, put forward by the [illegible], could be substantiated, as will be easily seen from the experimental portion of the paper

Experimental

One hundred grammes of the dried and powdered plant were extracted with warm dilute hydrochloric acid (2 per cent) The extract was dirty red in colour and was decolorized with animal charcoal This was then divided into four portions, viz, (*a*) original solution, (*b*) neutralized by caustic soda, (*c*) neutralized by sodium carbonate, and (*d*) neutralized by ammonia Nos 2, 3 and 4 gave curdy white precipitates which were filtered off and separately dissolved in dilute hydrochloric acid All these solutions as well as the original liquid were tested with alkaloid reagents The original solution as well as the filtrates from the precipitates mentioned above (after being rendered acid with hydrochloric acid) gave faint reactions of alkaloids,

but the hydrochloric acid solutions of precipitates Nos 2, 3 and 4 gave no reactions whatever These experiments show that the cuidy precipitates mentioned by the previous workers do not contain any alkaloid, but are purely inorganic in character (hydroxides and carbonates of calcium, magnesium and aluminum)

One hundred grammes of the dried plant were exhaustively extracted with distilled water, and the filtered extract treated with lead acetate The precipitated lead compound was filtered off, washed with water, and decomposed in aqueous suspension by hydrogen sulphide After removal of the lead sulphide by filtration, the mother liquor was evaporated, when a brown syrup was obtained yielding a greenish black precipitate with ferric chloride and consisted mostly of tannins It did not respond to any alkaloid reagents The mother liquor from the above lead acetate precipitate was also treated with hydrogen sulphide to remove the excess of lead and the filtrate from it evaporated, when a brown sticky substance was obtained having a strong sugary smell and reducing Fehling's solution very readily This substance gave only faint tests of alkaloids

One hundred grammes of the dried plant were extracted with water and to the clear aqueous extract a solution of tannic acid was added and the resulting precipitate filtered off and washed with water It was then triturated with freshly precipitated lead carbonate in a mortar and the mass reduced by gentle heat to a dry powder On extraction with alcohol and subsequent evaporation of the solvent a light brown gum was obtained which did not give any alkaloid reaction

One kilogram of the powdered plant was exhaustively extracted with distilled water and the extract evaporated to dryness The dark brown amorphous powder that was obtained smelt strongly of sugars and weighed 290 g This was then successively extracted with organic solvents in the following order —petroleum ether, chloroform, ethyl acetate and alcohol The extracts of the first three solvents were negligible in amount and contained nothing else except a little wax and traces of organic acids The alcoholic extract weighed 2 3 g and consisted mostly of sugars An acidified solution of this gave the following reactions with the alkaloid reagents mentioned below phosphomolybdic acid—a yellowish white turbidity, phosphotungstic acid—a slight white precipitate, Meyer's reagent—a trace of a yellowish white precipitate, Wagner's solution—a faint brown coloration, Dragendorf's reagent—a slight brown precipitate All other alkaloid reagents produced no change

One hundred grammes of the dried and powdered plant were then separately extracted with the following organic solvents and the solvents evaporated chloroform, ethyl acetate and alcohol The results are shown below —

Chloroform extract (4 2 per cent) Deep green semi-solid wax smelling strongly of chlorophyll Dilute hydrochloric acid extract of the substance on treatment with alkaloid reagents indicated their presence in minute traces

Ethyl acetate extract (1 1 per cent) Soft green wax with properties similar to the above

Alcoholic extract (13 2 per cent) Dark brown powder smelling strongly of sugars Did not crystallize under any condition Fehling's solution was strongly reduced and the substance was acidic to litmus Neutral ferric chloride did not produce any coloration or precipitate, but calcium chloride and lead acetate both produced curdy brown precipitates Acidified solutions of the substance indicated the presence of an alkaloid in minute traces

Examination of the ash The dried plant on ignition left 20 9 per cent of total ash of which 6 7 per cent was soluble in water and contained calcium, magnesium and potassium sulphate and traces of chloride The insoluble ash contained mainly silica and alumina

Estimation of sugars The dried plant was found to contain 8 2 per cent of only reducing sugars estimated in terms of glucose

One of the authors (G P P) wishes to express his indebtedness to the Kanta Prasad Research Trust of the Allahabad University for a scholarship which enabled him to take part in the investigation

Ind Jour Med Res, XX, 2, *October*, 1932

FILARIASIS IN NORTH TRAVANCORE

BY

M O T IYENGAR,

Medical Entomologist, Travancore

[Received for publication, September 27, 1932]

THE writer has found that the type of filarial infection occurring in two adjacent coastal taluks in North Travancore, namely, Shertalai and Ambalapuzha, is different from the infection observed in other parts of India Firstly, the microfilariæ found in the human carrier in this area are entirely different from those of *Wuchereria bancrofti* Cobbold Secondly, the chief transmitter of the infection is *Mansonia* (*Mansonioides*) *annuliferus* Theob , consistently negative results were obtained with *Culex fatigans* Wied , both naturally and in experimental infections

The microfilariæ observed in human carriers in Shertalai and Ambalapuzha, in common with those of *W bancrofti*, have sheaths and exhibit a definite nocturnal periodicity But they are distinct from microfilariæ of *W bancrofti* in general appearance, in size and in the arrangement of the nuclei These microfilariæ are much shorter (the average length being about two-thirds that of those of *W bancrofti*) They have at the anterior end a clear area devoid of nuclei which is much larger and more pronounced than in *W bancrofti* The position of the anal pore is marked by a clear break in the nuclei and a further characteristic is the presence of scattered nuclei distributed nearly as far as the extreme tip of the tail In many ways, these microfilariæ from Shertalai and Ambalapuzha are similar to those of *Filaria malayi* Brug, 1929, described from the Malay Archipelago Further work would determine whether the Shertalai microfilariæ are identical with *F malayi* or whether the two are distinct

In Travancore, both types of filarial infections occur In urban areas on the south, like Trivandrum, the infections are those of *Wuchereria bancrofti* In rural sandy coastal areas on the north, like Shertalai and Ambalapuzha, the infections are of the other type in which the microfilariæ are similar to those of *F malayi* Brug In the former area, *Culex fatigans* is a common species and it acts as an efficient intermediate host both in nature and under conditions of experimental infection

In the latter areas, *Culex fatigans* is sparse and this species has not been found infected, experimentally or in nature Several infection experiments were carried out with laboratory-bred *Culex fatigans* from Trivandrum, which were sent to Shertalai and fed on human carriers there In over 800 examinations, none of the specimens showed any developmental phases of the worm These results show that although *Culex fatigans* is very susceptible to infection with *W bancrofti*, it is entirely refractory to infection with the Shertalai microfilariæ On the other hand, *Mansonia annulifera*, which is a fairly common species in Shertalai and Ambalapuzha has been found to have a high natural infection rate (26 per cent in an examination of over 900 specimens) Laboratory-bred specimens of *M annulifera* fed on human carriers in Shertalai, readily took the infection, the filaria larvæ developed rapidly and reached the final proboscis stage in 11 days This species appears to be the main transmitter of this infection

Further studies are in progress on different aspects of the question The points briefly touched upon in this preliminary note would be dealt with in fuller detail in a later communication

REFERENCE

Brug, S L (1929) . *Filaria Malayi* n sp Parasitic in Man in the Malay Archipelago *Trans F E A T M, 7th Congress*, Dec 1927, **3**, pp 279–298

Colonel Sir RONALD ROSS

OBITUARY

Colonel Sir RONALD ROSS, K C B, K C M G, F R S, LL D, I M S (*retd*), (1857—1932)

'Let us now praise famous men, for their work continueth, greater than their knowing' —Kipling

With the death of Sir Ronald Ross on 16th September, 1932, India has lost one of the greatest of her sons Ross, with Manson, Laveran and Koch, was one of that great group of pioneers who founded the modern science of tropical medicine, and he was the last survivor of those famous workers The strides which have been made in tropical medicine, since Laveran discovered the malaria parasite in 1880, have probably been greater than in any other branch of medical science The majority of this development has taken place since the epoch-making discovery by Ross in 1897 that the mosquito transmitted malaria

Every inhabitant of the tropics, whether trader or missionary, soldier or sailor, engineer or doctor, fisherman or agriculturist, miner or explorer, mother or daughter, owes a debt of gratitude to this great scientist Many individuals of unborn generations will owe their existence to his discovery

If one considers the statement of that great physician Osler 'if a census were taken among the world's workers on disease, the judgment to be based upon the damage to health and direct mortality, the votes would be given to malaria as the greatest single destroyer of the human race', it is not too much to say that Ross's discovery was one of the greatest, probably the greatest, made in medicine in the last half century It has saved innumerable lives and has made practicable the development of large areas of the tropics, which were previously uninhabitable A striking tribute, among many, was paid to the value of his discovery by that famous tropical sanitarian, General Gorgas, who said, 'It seems to me not extreme, therefore, to say that it was your discovery that enabled us to build the Canal at the Isthmus of Panama'

The benefits which India, with her great incidence of malaria, reaps, and will reap, from Ross's great discovery, should always keep his

memory green in this country A Gate of Commemoration was opened in 1927 by Lord Lytton, the Governor of Bengal, at the Presidency General Hospital, Calcutta The doors of the gate bear a bronze medallion with Ross's effigy and the inscription —'In the small laboratory 70 yards to the south-east of this gate, Surgeon-Major Ronald Ross, I M S , in 1898 discovered the manner in which malaria was conveyed by mosquitoes' The Ross Field Experimental Station for Malaria at Karnal, Punjab, was instituted in 1926 by the Indian Research Fund Association to continue those researches in malariology with which Ross's name will always be associated

India can proudly claim Sir Ronald Ross especially as her own for he was born at Almora in the Kumaon Hills, on 13th May, 1857, of a family with many associations with this country He spent 18 years as an Officer of the Indian Medical Service and it was in India that he made his famous discovery

Sir Ronald Ross received his medical training at St Bartholomew's Hospital and he entered the Indian Medical Service in 1881 His early inclinations were towards literature, music and mathematics, and it was only after several years' service in India that he turned his mind seriously towards medical research During leave in England in 1888, he obtained the newly instituted Diploma of Public Health and took a course of bacteriology with Professor Klein These studies were the foundation of his expert knowledge of microscopy, which proved so useful to him in his later work

As the result of his observations in India at this time, he wrote an essay on malaria for which he was awarded the Parkes Memorial Prize on his return to England in 1895 In this thesis he put forward evidence against the generally accepted theory of the period, that malarial infection was caused by miasma arising from swampy areas As a result of this work he came in contact with Sir Patrick Manson, who demonstrated to him Laveran's malaria parasite, about which he, as well as many other workers, still maintained a considerable degree of scepticism

He returned to India filled with enthusiasm for further research work on malaria His chief object was the investigation of the theory that mosquitoes were responsible, in some manner, for the spread of malaria, a hypothesis which had been suggested in 1883-84 by King, Laveran and Koch, and of which Manson was a strong supporter

It is very difficult nowadays to appreciate the magnitude of the task which confronted Ross when he returned to India in 1895 and rejoined his regiment at Secunderabad At that time scientific medicine, as known at present, was in its infancy and medical protozoology or entomology had hardly been born Except for the discovery by Kilborne and Smith in 1893, that bovine piroplasmosis was transmitted by ticks, no one had dreamt of the spread of protozoal diseases by the bites of arthropods At this time only 4 species of mosquito had been recorded from India, while now nearly 300 are known, and Ross had no entomological training and no literature to guide him when he set out to explore the uncharted seas of the transmission of disease by these insects

Ross started to test Manson's tentative suggestion that the disease might be transmitted by drinking-water contaminated by mosquitoes which had fed on malarial patients, but his experiments met with no success

At this time the nature of the gametocytes of the malaria parasite was unknown and the flagellum was believed by some workers to be an independent organism and by others a degeneration product produced during the dying stages of the parasite Ross collected innumerable mosquitoes and after feeding them on malarial patients dissected them His first discovery was that exflagellation of the male gametocyte could take place in the stomach of a mosquito fed on a suitable case He continued his investigations, stimulated by Manson's enthusiastic support and his dictum—' follow the flagella '

The chase was a long one Through $2\frac{1}{2}$ years of unceasing toil, discouraged by heart-rending disappointments and technical difficulties, and hampered by cholera epidemics and the exigencies of military service, with indomitable perseverance he struggled on, sustained by the counsel and sympathy of Sir Patrick Manson About noon on 20th August, 1897, in the heat of an Indian summer, he saw for the first time the developmental stages of the human malaria parasite in the stomach-wall of an Anopheline mosquito, and thus opened up a new era in the history of disease transmission

At this time military duties interrupted his work, and not until the following year, when he was placed on special duty at Calcutta, had he an opportunity of completing his research In July 1898 he

was able to telegraph to his friend, supporter and confidant, Sir Patrick Manson, that he had proved the mosquito cycle of the malaria parasite by his experimentation with bird malaria and the Culicine mosquito In the years which have elapsed since he made his discovery under such trying conditions, very little of outstanding importance has been added to his original description

The details of the mosquito-malaria cycle are so extraordinary that they must have sounded like a fairy tale to the scientific world of the day, yet they were so convincing that, when Manson announced the discovery at the Annual Meeting of the British Medical Association in Edinburgh in July 1898, the members rose and cheered Ross's results were quickly confirmed, and the enormous value and scientific importance of his discovery recognized

Although Ross sometimes spoke of the luck which attended his discovery, one can not but think that a lesser man not possessing his grit, energy, determination and ability, would have given up in despair before the enormous difficulties which beset his path

Sir Ronald Ross left India in 1899 and was quickly appointed to a teaching post at the newly-formed School of Tropical Medicine in Liverpool While in this post he took part in many expeditions to the tropics in connection with malarial investigations and prevention The chief of these were to the West Coast of Africa, to Ismailia, to Greece and to Mauritius

There is no branch of malariology which has not felt his influence During the 13 years in which he was at Liverpool he taught and inspired many workers, not only in connection with malaria, but also in relation to many other tropical diseases

At [illegible] of the Great War, he acted as Consultant in [illegible] of Colonel He visited on a ship which was Honorary Consultant in [illegible] He was for many [illegible] Committee and of Fund Association, in collaborators in the

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TAYLOR, G (1917) *Ind Jour Med Res*, **5**, No 2, p 497

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Ann Inst Pasteur	*Jour ...*
Arch Schiffs u Trop Hyg	*Jour*
Brit Med Jour	*Jour*
Deut Med Woch	*Lanc...*
Ind Jour Med Res	*Med ...*
Ind Med Res Memoirs	*U ...*
Ind Med Gaz	*Tra*
Jour Exper Med	*Tr...*
Jour of Hyg	*Z...*

Some years later a public subscription was started which resulted in the foundation of the Ross Institute for Tropical Diseases at Putney Heath, London This institution was inaugurated by H R H The Prince of Wales on 15th July, 1926, with Sir Ronald Ross as Director-in-Chief

Unfortunately he had a paralytic seizure in 1927, but even this did not stop his unbounded energy and he continued at work, more especially in his mathematical studies, until a few months before his death

Ross's genius manifested itself in many forms Although he will always be remembered for his medical researches, yet his work on pure mathematics and pathometry, more especially in relation to malaria, was of a high order His scientific achievements have won for him world-wide fame and have overshadowed his work in the field of art He was not only a musician of no mean ability but also a novelist and a poet Indeed at one time he seriously contemplated abandoning medicine for literature Of his poetry, Mr John Masefield, the Poet-Laureate, has spoken in most eulogistic terms

Sir Ronald was always a staunch friend and an enthusiastic helper to research workers who consulted him The campaign for improving the emoluments, and for the state endowment of research workers received his strong support

Sir Ronald Ross was made F R S and F R C S in 1901 He was decorated C B in 1902, K C B in 1911 and K C M G in 1918 His great discovery gained for him the Nobel Prize for Medicine in 1902 and very many Governments and Universities awarded to him honours and degrees in recognition of the great benefits which his discovery has conferred upon humanity

All over the world, workers in tropical medicine, and more especially in malariology, will deplore the loss of a great scientist and his old pupils will also mourn the passing of a valued friend and teacher

Resolutions of sorrow at the death of Sir Ronald Ross and of condolence with his relations were passed at the meetings of the Scientific Advisory Board and of the Governing Body of the Indian Research Fund Association held in September 1932

J A S

Ind Jour Med Res, XX, 3, *January*, 1933

AN INQUIRY INTO SNAKE-BITE IN IRAQ*—*concld*

BY

NORMAN L CORKILL, M B , Ch B , F Z S ,
Sudan Medical Service
(*Late Iraq Health Service*)

[Received for publication, February 22, 1932]

Poisoning by *C cornutus*

Though celebrated and of evil reputation, this viper has a surprisingly small literature containing, as far as I know, no record of any human fatality Phisalix (1922) has summed up the net effect of the literature which shows that the venom is fatal to small animals and birds The characteristic viperine reaction at the site of injury develops One is led to feel that perhaps Herodotus was right when, in describing this snake, he said it was harmless Again, Anderson (1898) was not convinced of its dangerousness to man He quotes a case in which a man who was a professional snake-catcher was bitten between finger and thumb by a *Cerastes cornutus* that sprang three feet at him He took no remedial measures and did not suffer The same snake was made to bite a pelican on the leg, the bird died in thirteen minutes Whether the snake-catcher was immunized or not cannot of course be stated Calmette (1908) says the species frequently causes fatal accidents amongst barefooted Arabs and blacks He does not, however, adduce any specific instances The snake is an object of fear to the natives but so are quite many harmless snakes The toxicity of the venom, as far as I know, has not been worked out Phisalix (1922) quotes Calmette as giving its maximum yield in milligrams of dried venom as 19 to 27 On existing data its dangerousness cannot be assessed The two cases mentioned below are not strict evidence but nevertheless I produce them here and am personally inclined to accept them as *bona fide* cases of *C cornutus* fatalities as they are not the type of fabrication that Arabs evolve when yarn spinning An Arab describing an imaginary snake's head might talk of its having hair or horns or a crown but he would never

* The first half of this paper has been published in the October 1932 number of the *Journal*

dream of confining himself to such an unspectacular particular as that 'its head was like a triangle' Similarly, when an uneducated Arab describing a snake which he has never seen before mentions all its salient characteristics correctly and moreover mentions none that is incompatible, it is fair to accept his story as truth

CASE XVIII —*Bite by* C cornutus *with Fatal Result Mosul* Details communicated by Dr W Corner, Civil Surgeon, Mosul, in 1929

On 15–7–29 a Syrian doctor was sent from Mosul to inquire into the reported death of a man at the hill village of Dera Gidjnik near Dohuk north of Mosul It was rumoured that it was a snake bite fatality but there was a suspicion that homicide might be a more correct diagnosis

The doctor returned satisfied that it was a case of snake bite The snake which had been killed and buried was described by the relatives of the victim as having horns It was two and a half feet long, having been measured in hand spans, and had a thick body and a head broader than its body The bite had been on the little finger and severe swelling had subsequently spread up the arm Death occurred in a little over twenty four hours after the bite

Commentary —Questions about the Horned Viper in these hills invariably produced the reply that they were known of but that no one had ever seen one The natives of Dera Gidjnik from whom the details of the snake and bite were collected could scarcely be expected to have naturalists' accurate knowledge of the size, shape, and detail of a snake they had never seen, coupled with a knowledge of the clinical course of its poisoning Therefore as all points mentioned by them suggest definitely a viper and moreover the Horned Viper in particular by virtue of horns and 'a head broader than the body' I consider the case as acceptable This is the first record of the snake east of the Euphrates

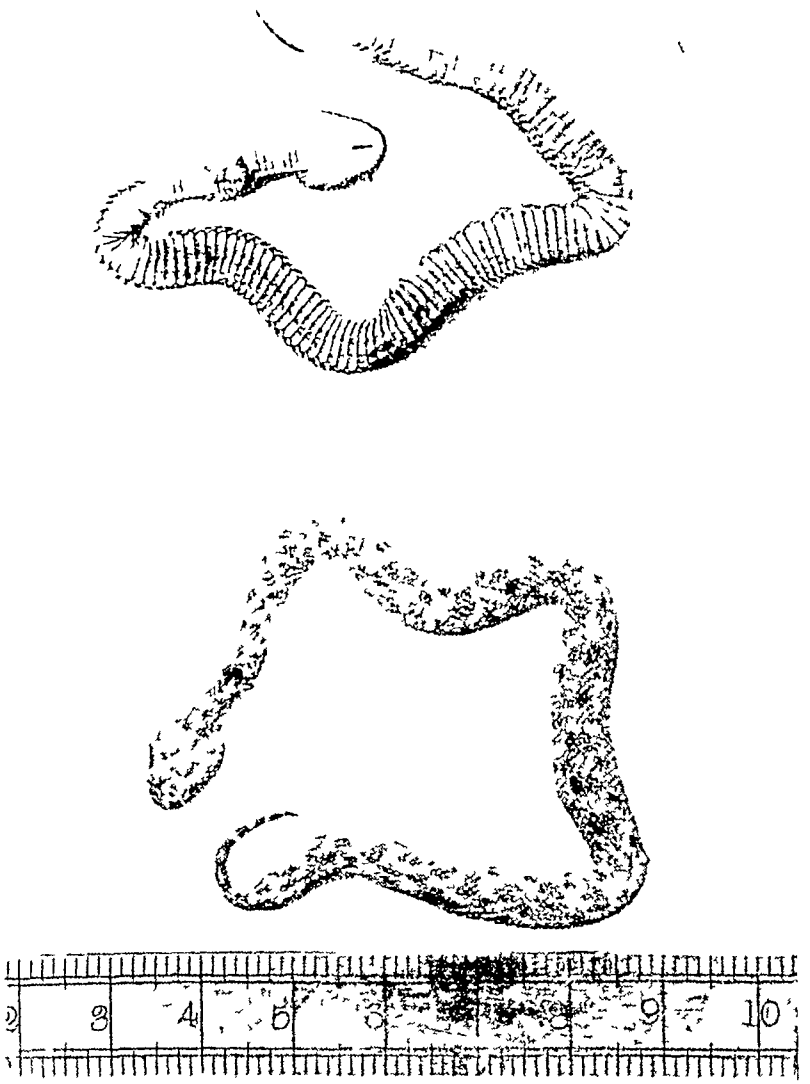
Death in just over twenty-four hours points to either a large dose of venom or a very potent one It has been noted already that the yield is small (*vide supra*) in this species, so it would appear that the venom of *Cerastes* is powerful A length of two feet six inches means a full grown snake No hæmorrhages are recorded and had such spectacular symptoms occurred they would certainly have been mentioned, so one may assume that, accepting the venom as being typically viperine, its hæmorrhagin content is small Death was probably due to cardiac depression, possibly by central action, possibly by local, probably by both In small animals (Phisalix and Anderson) death appears to be induced largely by intravascular clotting as in poisoning by other viperine genera

CASE XIX —*Bite by* C cornutus *with Fatal Result Baghdad* The following story was told to me first by a medical student in 1928 His informer had been the family nurse I subsequently heard the same story several times from different sources quite out of contact and association with each other

During the Turkish time (i e , before the British took Baghdad) a house was being built in Baghdad and while the work was proceeding a snake was encountered with a triangular head A Sheikh was brought to catch it (a common procedure in Baghdad nowadays) This he did and was bitten by the snake He died shortly afterwards Pieces of wood were used to bury him with so that there would be no danger of any person getting poisoned through handling the body

Commentary —For me, mention of a triangular head not only instantly firmly establishes the snake spoken of as a *Cerastes* (see Plate XXV) but gives the story the stamp of authenticity Again it is a record from a country east of the Euphrates Death shortly afterwards is hard to interpret, it may mean minutes, hours, or days I am inclined to believe death to have been within say two hours, if not immediate, because the astonishing and unusual burial precautions suggest that the end was powerfully and impressively tragic and dramatic Did he die in convulsions it may be wondered, a thrombotic death?

It is felt that the evidential value of the foregoing two cases does not perhaps attain to a standard of perfection but in view of the scarcity of data on poisoning by this snake it has been thought of interest to record them Should future research on the venom confirm these cases, it will mean that *C cornutus* must rank as a very poisonous species As, however, it is fairly common in North Africa



Cerastes cornutus, the Horned Viper
(*By Courtesy of the Royal College of Medicine, Baghdad*)

Plate XXVI

Echis carinatus, the Saw-scaled Viper

(The photograph is of Sudanese specimens They differ from Mesopotamian types in no essential particular)

(By Courtesy of the Director, Wellcome Tropical Research Laboratories, Khartoum)

and no case records are available it is safe to say that it is not a very dangerous snake as regards man Possibly it is non aggressive under natural conditions

Poisoning by *E carinatus*

Acton and Knowles (1921) tabulate the estimated fatal dose for man as 5 0 milligrams of the dried venom, and the approximate dose given at a bite as 12 3 milligrams This is over twice the fatal dose The venom is thus given by these workers as being three times as toxic as the venom of *Naja naja* Wall (1928) estimates the lethality of *E carinatus* as 20 per cent There is always the chance with this snake of a sub-lethal dose being inoculated and this accounts for the apparent discrepancy in that cobra-bite is claimed to have a 30 per cent mortality although its venom is only a third as toxic It can, however, administer ten lethal doses at a bite Compared with *V russelli* it (*E carinatus*) is eight times as toxic but can only administer the same number of fatal doses that is, two In point of fact it actually appears not to have as high a lethality as *V russelli* (or *V lebetina*, i e , 33⅓ per cent as quoted previously) The reason probably is that being a smaller snake it has more difficulty in driving home an efficient strike than have the more powerful and much bigger members of the genus *Vipera* Its venom differs mainly from that of *V russelli* (and therefore presumably *V lebetina* also) in that there is a much larger proportion of hæmolysin and hæmorrhagin present Wall in his review of the literature does not mention the occurrence of fibrin-ferment, leucolysin, or cytolysins as in *V russelli* venom As one would therefore expect the clinical picture is dominated by shock, depression, and hæmorrhages from the wounds, nose, gums, bladder and bowels Symptoms are frequent also, due to hæmorrhages into internal organs I have not found any reference to thrombotic symptoms Three typical cases are given in detail by Wall (1928) in his ' Poisonous Terrestrial Snakes of India ' but as they resemble the cases recorded below in which the snakes were identified there is no point in discussing them here Four cases in which the snake was identified, and of which one terminated fatally are detailed below A fifth case, also fatal, in which the snake though not identified was almost certainly an *Echis*, is also included in the series The mortality for a series of five cases is thus two or 40 per cent, probably too high an index, the series being small

Case XX —*Bite by* Echis carinatus *with Fatal Result Diwaniyah* Details of a case previously recorded by Sinderson (1924)

T A P , a healthy well built Indian aged twenty eight, and who was Station Master at Imam Hamza on the Middle Euphrates near Diwaniyah, was bitten on the ball of the right thumb at 8 P M on 28-8-23 The snake was secured and subsequently identified as an *Echis carinatus* It was sixteen inches long A tourniquet was applied at once Later (5½ hours after the bite) pronounced swelling of the arm and uncontrollable oozing of blood from the two punctures brought the patient to Diwaniyah Civil Hospital Here the wound area was incised and potassium permanganate was rubbed in The arm as far as the elbow was very swollen, excruciatingly painful, and exquisitely tender , it was of a livid hue The sanious oozing persisted and the patient was coughing up blood stained material The next day at 10 A M (14 hours after the bite) these symptoms had become aggravated and

hæmaturia and bleeding from the gums had made their appearance Later he was placed on the northward bound train and arrived in Baghdad after a six to seven hours' journey on the morning of the 30th He was admitted to the Railway Hospital there under Dr H C Sinderson

This would be about 36 hours after the bite On admission the patient was noted to be greatly collapsed and the wounds were still bleeding Shortly after arrival he passed a stool containing much unaltered blood The pupils were dilated The pulse rate was 110, the temperature was 99°F , and the respirations were 38 Swelling above the elbow was not marked but the axillary glands were enlarged and tender No antivenin being available, treatment was perforce symptomatic Styptics were applied to the wounds but proved ineffective Horse serum and calcium chloride were given intramuscularly and saline was administered per rectum The condition passed into one of extreme collapse at which stage a cannula was tied into the median basilic vein of the unaffected arm and by this route cardiac stimulants in hypertonic saline were administered periodically Hæmorrhages from the original wounds, the gums, the bladder, and the cannula insertion site persisted and profound collapse and bloody expectoration dominated the situation till death at 11 15 A M on the 31st (63 hours after the bite)

Commentary —The snake was by no means a large one, sixteen inches being rather below the average of specimens encountered The tourniquet is said to have been applied on the receipt of the bite This in itself might mean anything up to ten minutes and as it was applied by a lay person one may assume that it was most probably placed round the wrist or forearm Its efficacy in this case may therefore be doubted The severity of the reaction, a matter of some six hours only after the bite, bears witness to the potency of the venom The coughing up of blood stained sputum probably derived from the gums, at this early stage, is also notable Although hæmaturia is not recorded till fourteen hours after the bite it is probable that examinations of the urine microscopically would have revealed the presence of red blood corpuscles much earlier I have been unable to find any record of melæna in a case of snake bite in any instances other than those in which the snake concerned was an *E carinatus* A supra lethal dose of venom was apparently absorbed and fixed here in a short time and the cytolytic type of death was probably inevitable after the first minute or so after the bite had passed

Cases XXI and XXII —*Two cases of bite by* E carinatus *followed by Recovery Diwaniyah* These were reported by Dr McLeod, Civil Surgeon, Diwaniyah, in April 1924

Two policemen in Diwaniyah had two snakes in a sack and in the course of playing with them were bitten The snakes were subsequently identified as *E carinatus* Both men were admitted to Diwaniyah Civil Hospital, had similar symptoms and recovered The case of the more seriously affected man is described first and in more detail

Case XXI —S K , a healthy Arab adult, was admitted to the hospital at 2 15 P M on 9-4 24, fifteen minutes after having been bitten on the right index finger Pain was complained of and the hand was swollen to the wrist The site of injury was incised and potassium permanganate rubbed in

At 5 30 P M (3½ hours after the bite) the patient collapsed and the swelling had reached the elbow The pulse was irregular and the temperature was 98 4°F Brandy, strychnine and rectal salines were administered The next day (18 hours after the bite), the swelling had reached the shoulder, the axilla was painful, and there was a tender spot below the umbilicus At 10 A M (20 hours after the bite) collapse recurred The ante cubital fossa was opened and calcium chloride and saline were given intravenously Improvement was immediate Blood oozed continuously from the original incision and the swelling progressed until the left pectoral region was involved The patient complained mainly of pain below the umbilicus An enema procured an evacuation suggestive of constipation only This gave abdominal relief The urine was tested for blood , none was detected Anything swallowed was vomited The evening temperature (28 hours after the bite) was 99 5°F and the pulse is recorded as having been satisfactory The next day, that is, the 11th (42 hours after the bite) there was an increased loss of blood from all incisions and blood was being spat out The swelling involved both sides of the chest completely Half an ounce of sodium sulphate was given The evening temperature (52 hours after the bite) was normal On the 12th (66 hours after the bite)

the oozing of blood was less except from the original incision which was bleeding more freely than ever The temperature was normal and the pulse regular though weak From this time onwards the condition improved gradually, hæmorrhage from the original wounds alone proving troublesome By the 17th (8 days after the bite) the patient was greatly recovered although still suffering from general debility and severe anæmia Both of his arms showed patchy ecchymoses

Commentary —The elapse of fifteen minutes before first aid probably meant that little advantage if any accrued from the conventional incision and rubbing in of permanganate Abdominal pain and tenderness is not an uncommon thing in *Echis* poisoning although its significance is hard to assess It occurs in cases where melæna is recorded and also in those in which no such condition has been noted In a case reported by C H Reinhold (1910) severe abdominal pain was a salient symptom and there was no melæna At autopsy a large retro peritoneal hæmorrhage was present It was ascertained that the spleen and kidneys were not the source of the hæmorrhage, the omentum, however, was anæmic Abdominal pain might be the accompaniment of many intra abdominal reactions but in view of the fragile support given by the adjacent tissues to the mesenteric vessels it seems reasonable to suspect that intra mesenteric and retro peritoneal hæmorrhages may be the lesions concerned Cases in which melæna is present closely resemble in their clinical features cases of Henoch's Purpura in which sero hæmorrhagic exudations into the gut wall take place (Tidy, 1925), melæna being a noteworthy clinical accompaniment It is of interest that it is recorded that 'the urine was tested for blood, none being found' The test applied is not stated but I have myself seen red corpuscles under the microscope in specimens which did not show a detectable opacity on applying the usual simple heat test for albumen The presence of red blood corpuscles in the urine in increasing quantities up to a point may probably always be demonstrated microscopically in *Echis* poisoning The markedly severe and diffuse tumefaction is characteristic as are also the patchy ecchymoses not confined to the area of initial injury

Case XXII —This, the second policeman, was a healthy adult Arab and was also bitten on a finger No marked swelling occurred On the second day (18—30 hours after the bite) he spat blood, this stopped the following day (42—54 hours after the bite) From this time (42—54 hours after the bite) until the seventh day there was great pain in the opposite arm which the patient could not move There was no swelling in it and it is not recorded whether or no it was tender Recovery was complete

Commentary —The amount of venom inoculated was apparently small as the local reaction was so mild Such being the case, the severe bleeding from the nose for twenty four hours and the hæmorrhage into the muscles of the opposite arm, for I can see no other reason for the pain and resulting inability to move the arm, attest the very high hæmorrhagin content in *Echis* venom

Case XXIII —*Bite by* E carinatus *followed by Recovery Diwaniyah* Details communicated by Dr Lanzon, Medical Officer, the Railway Hospital, Baghdad, in 1929

B J, a healthy Arab railway coolie aged about 25, was walking on the railway line at Khan Jadwhal in the Diwaniyah area at 11 A M on 8-5-27 He suddenly felt a bite on his toe (third left) and looking down saw a small snake This he at once killed He then ligatured his ankle tightly with a cloth He was sent to Baghdad by train arriving twenty four hours later

On admission (24 hours after the bite) no punctures could be seen but the foot was swollen The under surface of the third left toe was scarified until it bled and potassium permanganate was rubbed in The ligature was then removed A telegram was sent requesting the despatch of the snake On arrival it was identified as an *E carinatus* At 6 P M that evening (31 hours after the bite) there was slight epistaxis By 10 A M the next day (47 hours after the bite) this had increased and moreover hæmaturia and melæna had made their appearance Intravenous salines, calcium chloride, and adrenalin were given and at noon and in the evening antivenin was administered On each of these occasions 40 c c were given That evening (55 hours after the bite) the temperature was recorded as 101 6°F, it rose to 103 6°F on the following day and dropped to normal on the morning of the 14th (sixth day after the bite) Bleeding persisted longer, epistaxis ceasing on the 16th (eighth day after the bite) and hæmaturia not disappearing till the seventeenth (ninth day after the bite) A month later (thirty nine days after the bite) the patient was seen again when the leg, though still a little tender, was noted to be functioning without disability

the case it should always be the endeavour of the responsible medical attendant to ascertain what evidence may exist as to the cause of a reputed snake-bite accident and should a favourable deduction be possible to impress its significance upon the patient in the most emphatic terms

Many instances are encountered in which a person has been walking barefoot at night and has suddenly felt a prick Great importance should be attached to the nature of the ground and surroundings and the immediate sensation as mentioned by the patient The presence of camel-thorn in the neighbourhood and no feeling beyond a prick suggest a thorn A diffuse pain or burning sensation throughout the foot or arm suggest a scorpion or poisonous snake A prick without such sensations and where thorns are absent suggests a harmless snake

Enhydrina schistosa poisoning may occur only in fishermen or others on the sea-coast or rivers up to say sixty miles from the sea *N morgani* poisoning may occur all over Iraq *V lebetina* poisoning may occur only in the quadrant of Iraq comprised by the country north and east of Baghdad *Cerastes cornutus* poisoning is possible throughout Iraq but probable only down the Euphrates valley and west of it *Echis carinatus* poisoning may occur only in the Diwaniyah area of the Middle Euphrates valley

Symptoms due to apprehension of the result rather than from toxæmia due to the inoculation may be confirmed by the absence of a powerful heart-beat, present in cobra poisoning, and by the absence of severe local reaction present in viperine poisoning In poisoning from *Enhydrina schistosa* and *Naja morgani* the following may be expected local reaction at the site of injury not very severe, numbness or tingling rather than pain, weakness of the legs, ptosis, failure of the voice, dribbling of saliva, drooping of the head, falling of the jaw, inability to swallow, stertorous respiration, strong heart-beat, and death, if it is going to occur, probably within ten hours, it may be heralded by convulsions Poisoning from *V lebetina* will be characterized by severe and gangrenous reaction at the site of injury with diarrhœa, hallucinations, great vaso-motor depression jaundice, and necrotic indolence of affected tissues *Cerastes cornutus* presents more of a diagnostic difficulty but severe reaction at the site of injury may be expected and also chronic sepsis, initial vaso-motor depression it is also reasonable to expect The diagnosis of bite by *Echis carinatus* appears simpler Red blood corpuscles in the urine six hours after the bite, early and very severe hæmorrhages from mucous surfaces particularly from the bowel, and abdominal pain and tenderness are characteristic

Scorpion stings do not produce hæmorrhagic symptoms and are characterized by vaso-motor depression and muscular contractions, further there is no local necrosis

An examination of the site of injury may reveal a thorn, a fang embedded in the wound, a single puncture, two larger punctures each succeeded by several

smaller, or two large punctures only, or two or four rows of sub-equal punctures A thorn removed and shown to the patient should make all concerned happy An embedded fang should be examined to see if the canal that traverses it is completely roofed in or whether it is more of the nature of an almost completely folded in groove This latter condition would mean that *Naja morgani* was concerned and the definite closed canalization would indicate one of the three vipers A single puncture that does not ooze blood is probably a scorpion sting, but by no means necessarily so, for a snake may quite possibly strike inefficiently and drive only one fang home Two larger punctures followed by several smaller ones and possibly with two rows of small punctures intervening and extending further back ideally represent a cobra-bite whereas two large punctures with the two intervening rows of palatine teeth marks would mean a viper and four rows of sub-equal punctures would mean an aglyphous or opisthoglyphous snake Only too frequently perhaps an examination of the site of injury will suggest little of value On the other hand there are occasions when the findings may save much time and trouble

The Treatment of Snake-Bite in Iraq

A consideration of the treatment of snake-bite in Iraq should presumably involve a discussion of the theory of such treatment, a criticism of that applied in the twenty-six cases detailed above and thirdly the formulation of a simple scheme of treatment in cases where the snake is *E schistosa* or *N morgani*, *V lebetina*, *C cornutus*, *E carinatus*, or in cases where the poisoning is undiagnosable except as from snake-bite

An examination of the theory of snake-bite treatment leaves one in Iraq with a sense of helplessness in the presence of a case of snake-bite in which a lethal or supra-lethal dose has been supposedly inoculated, for no specific antivenins are available The literature on first aid methods approves unanimously no measure at all but usually, after great debate, makes qualified mention of the conventional ligature, incision, and chemical destruction *in situ* of any venom as yet unabsorbed

To criticize the treatment accorded to the above cases and to point out how certain procedures may have counted against the patients in their fight against the poisonings is more or less easy and possibly may appear ungracious It should be emphasized, however, that snake-bite is economically negligible, that medical life is very practical and the study of the every-day economic necessity must necessarily shelve the distant rarity, that the literature on snake-bite treatment is mostly split about and tucked away in the less common journals and more expensive books, that the average manual on tropical diseases does not treat the subject profoundly and that even when more detailed information is consulted by the harassed medical officer he finds generally, and perhaps necessarily so, debate and citation of authority, argument, and experiment rather than clear, concise, and convincing

instruction I am of course fully aware of the masterly work and summaries of Acton and Knowles, and of F Wall, they are rarely, however, immediately available to the medical officer who meets his case in the field None the less it may help to point out harmful procedures which could be quite easily avoided

A simple scheme of treatment for snake-bite cases in Iraq is detailed below but it must be confessed that its review fills one mainly with a sense of inachievement One can do little curatively, one may help nature, however, by minimizing the effects of the venom

Theory of Snake-Bite and its Treatment

In the absence of specific antivenins the aims will be to prevent or control absorption and fixation of the venom, to eliminate or destroy *in vivo* any as yet unfixed, and to counter the symptomatic evils resulting from its action

The nature and degree of the poisoning vary by reason of many factors There is the toxicity of the venom to be considered in conjunction with the average yield, from these may be estimated the number of fatal doses for an adult human of standard weight under optimum conditions of inoculatory efficiency Again, the amount inoculated will be governed by the healthiness of the venom glands and the integrity of the fangs A snake that has just engulfed a meal may be expected to have less venom available than a fasting one The maturity of the snake will again be a factor, the bigger the snake, the more the available venom will be The nature of the mouth-parts and the inoculatory mechanism is a further factor A sea-snake with its insignificant fangs and small gape cannot claim the efficiency of strike possessed by *Echis* with its long erectile ' needles ' and its gape of 180°, nor can a twenty-four-inch *Echis carinatus* vie with a sixty-inch *Vipera lebetina* for the latter has a bigger gape, longer and more powerful fangs, more momentum behind its strike and much more venom An adult of each of these two last species may inoculate in the neighbourhood of two fatal doses at a bite A drop or two lost in striking from the amount available will scarcely affect the result in *V lebetina* bite but in *Echis carinatus* bite it may mean the difference between life and death to the victim As regards the person bitten, many modifying factors are concerned, such as, protection partial or complete by clothes, boots, puttees, etc, whether or no the snake was jerked away at once or whether it remained attached to the site of injury, ' worrying ' or ' chewing on ', the nature of the inoculation, graze or puncture, and if the latter whether subcutaneous, intramuscular or intravascular, body-weight of the person bitten and his psychical reaction, and also his physical health The nature of the venom's physiological effect and whether first aid modified this in any way is the next point and lastly there remain the remedial measures subsequently adopted

Discussing the rate of fixation of venom in the tissues of the person inoculated, Acton and Knowles estimate an absorption time for *N naja* venom of ten minutes

and one of twenty to thirty minutes for that of *V russelli* on account of its thrombase content which tends to confine the venom to a certain extent to the vessels of the injured area These times may be taken to apply also to *N morgani* and *V lebetina* *E carinatus* has not been shown to contain a fibrin ferment and therefore may be assumed to have an absorption time comparable with *Naja* venom As regards fixation by the tissues *N naja* venom is reported by the workers just mentioned to be fixed slowly over a matter of hours whilst *V russelli* venom fixes with great rapidity Its fixation, moreover, implies gross damage by the killing of the endothelial linings of blood vessels Again, these characteristics may be applied respectively to *N morgani* and *V lebetina* *E carinatus*, in addition to being absorbed as quickly as *Naja* venom, has a larger hæmorrhagin content than *Vipera* venom and therefore probably causes a maximum of direct damage in a minimum of time

An appreciation of the foregoing is a helpful prelude to a consideration of what first aid and remedial measures should strive to attain and what they may hope to achieve First aid is directed to the limitation of absorption and the destruction of any venom that may be as yet unfixed Their conventional methods are by ligature, incision, excision, amputation, suction and phlebotomy, and by the injection of the specific antivenin when possible or failing that by the rubbing in or local injection of chemicals claimed to be destructive of venom *in vivo*

Ligature to be effective must obviously be applied before a lethal dose of venom has been absorbed if it is going to be of any value This gives a very small time margin to be played with Probably up to two minutes after the bite it is of marked value, up to ten minutes of some value and after half an hour of no value in *Echis* and *Naja* poisoning and of very slight value in *Vipera* poisoning If too late to be helpful it needlessly adds to the patient's sufferings A ligature should efficiently arrest the venous return but should not obliterate the arterial pulse Complete ligation is liable to cause mortification *per se* A ligature may only be applied round single bones, that is a phalanx, a humerus, or a femur An Esmarch's tourniquet or other elastic mechanism is desirable and should be loosened every twenty minutes for three minutes and then retightened In addition to being more efficient an elastic ligature is not so painful as an inelastic one

Incision may allow a small portion of poisoned blood to drain away but it also mechanically may serve to force more venom from the surface and site of inoculation into the tissues, further, in *Vipera* poisoning it lays open a larger area to the infection that is almost certain to eventuate

Excision may help in *Vipera* poisoning in which a certain amount of venom is locked up in the site of injury by thrombosis If ligature was later than half an hour after the bite it would, however, be a futile procedure The wound in any case may be relied upon to exhibit severe sepsis In *Naja* and *Echis* poisoning

no thrombase is contained in the venoms and absorption is rapid excision is, therefore, in such cases quite useless but if a ligature has been placed round a single bone within two minutes and the circumstances suggest that a lethal dose of venom may be locked up in the ligated area it is justifiable to amputate above the ligature without relaxing it Similarly, where *Vipera* is involved the time preceding ligation may be stretched say to twenty minutes and amputation (or excision) may still achieve good

If a ligature has been effectively applied and no antivenin is available some effort must be made to destroy or eliminate the venom contained in the ligated area The rubbing in of potassium permanganate crystals theoretically may destroy a certain amount of venom with which it comes in contact, particularly in thrombase-containing venoms Potassium permanganate, however, is escharotic to the tissues and the action of rubbing it in probably diffuses venom further by massage Injecting it into the site with an intramuscular needle may destroy a certain amount of venom , it will certainly cause tissue destruction Suction mechanisms applied to the site of injury, incised or untouched, cannot do any harm Incision of the main surface veins in the ligated area proximal to the bite and the withdrawal of two pints of blood, combined with the introduction of a similar or greater quantity of donor's blood or Bayliss's solution by an unaffected arm may help The mechanism is the washing out with blood of the poisoned area by means of the arterial pump It is probably of little value as the deep veins are not opened up It is debatable whether they should be , a stage little short of amputation or subsequently demanding amputation would soon be reached Surface veins opened should be selected as near the ligature as possible and should subsequently be ligated themselves with the strictest aseptic precautions, by understitching and tying over the superjacent skin The aim here is to prevent their developing into foci of sepsis and oozing hæmorrhages in viperine bites

There is nothing to be gained by discussing the use of real remedies, that is, specific antivenins, for in Iraq such do not exist Calcium is mentioned by Wall (1928) in his review of its use in snake poisoning as having been shown to abate the toxicity of cobra venom There are no other antidotes of proven value and further treatment is symptomatic unless one cares to classify blood transfusions as antidotal Blood transfusions seem to offer the only hope in certain cases of viperine poisoning in which a cumulative toxæmia combined with a grossly vitiated blood provide too great an onslaught for the body's natural defences to fight against The draining away of poisoned destroyed blood and the introduction of fresh blood replete with red cells and leucocytes may be expected to help

In cobra poisoning, artificial respiration should be kept up as long as the heart beats although it cannot be theoretically justified in the absence of antivenin

Should respiration be failing the administration of carbon dioxide and oxygen may be tried or the patient may breathe in and out of a bag thus increasing his carbon dioxide intake

In viperine poisoning, the evils to be countered are destruction of blood cells and of vascular linings, persistent hæmorrhages, destruction of liver and kidney cells, accumulation of katabolic and necrotic products of a toxic nature, great vaso-motor depression, gangrene, general sepsis, specific infections such as tetanus and malignant œdema, and generalized septicæmia Venesection and blood transfusions, calcium intramuscularly, hæmoplastin or normal horse serum, adrenalin, salines and hot packs and fluids, always having regard to the pulse, digitalin, strophanthus, pituitrin and frequent small feeds of coffee, soup, cocoa, tea, milk, etc , early amputation rather than late, polyvalent anti-streptococcal serum and specific sera when indicated all have their value Early stimulation and movement are bad as the venom is thus more quickly diffused Ammonia and alcohol as first aid measures may thus be classed as harmful Strychnine being cumulative is dangerous and better avoided , digitalin, pituitrin, and adrenalin are better and safer as stimulants and also have other valuable effects in addition

In the cases discussed above potassium permanganate treatment probably facilitated the advent of necrosis and sepsis in most instances and the amount of venom if any destroyed by it cannot have been appreciable Other practices that probably were harmful were activity and travelling immediately after the bite, and activity after a period of four days' inactivity following the bite In the former venom was probably diffused and fixed much more rapidly than it need have been and in the latter the rupture of injured vessels undergoing a reparative process initiated a fatal train of symptoms

Based on the preceding considerations the following scheme of treatment for cases of snake-bite in Iraq is formulated —

First Aid

If the patient is seen within ten minutes of the bite

(1) Secure absolute recumbency and rest

(2) If the situation of the bite permits apply a ligature by a rubber band or other elastic mechanism around the digit, upper arm or thigh concerned If the bite is on a digit apply a ligature around both digit and upper arm or thigh as the case may be The ligature should prevent the venous return but not the arterial feed Every twenty minutes the ligatures should be relaxed for three minutes and then retightened This may be continued for six hours

(3) Swab the surface of the injured area with strong potassium permanganate solution, iodine, or spirit, to remove any venom still lying on the surface Cover the site with a sterile or mildly antiseptic dressing

(4) Reassure the patient that the danger from snake-bite is vastly over-rated, that two-thirds of all cases bitten by the much feared Indian cobra recover, and that there is always a very good chance of a sub-lethal dose having been administered

(5) Secure the snake whenever possible Its identity may help in forestalling symptoms if a viper, and if it is a harmless snake the pointing out of its characteristics to the patient may prevent death from fright Moreover, its recognition as a harmless snake will save much discomfort from further medical and surgical measures by rendering them unnecessary

(6) Remove the patient with the minimum of movement to the nearest place where medical observation and attention can be applied

If the patient is seen more than ten minutes after the bite

Act similarly, but ligation is of value only if the snake is a *V lebetina* or unidentified and even then it is a useless measure if more than half an hour has elapsed

HOSPITAL TREATMENT

(A) Immediate

(1) Diagnose the case as cobra poisoning, viperine poisoning, doubtful, or not snake poisoning (include snake-bite from a non-venomous species)

(2) If one of the first three diagnoses is made secure a suitable donor for blood transfusions in view of possible eventualities

(3) Give an injection of polyvalent anti-streptococcal serum Dental and oral sepsis is common in snakes

(4) Commence intramuscular injections t d s of 2 per cent calcium chloride solution in two drachm doses This is of value in cobra poisoning (*vide supra*) and in viperine poisoning it replaces the calcium lost by hæmorrhage and counteracts the anti-coagulant effects of the venom

(5) If a ligature has been suitably applied within a reasonable time the surface veins may be opened as described above and an attempt made to drain away poison-containing blood Donor's blood or a suitable solution may be introduced by an unaffected arm

(6) Give a saline purge and later a simple soap enema to limit potential toxæmia and render the patient in a suitable state for the reception of rectal salines

(7) Secure tranquillity and rest till any venom absorbed may be assumed to be fixed , that is up to, say three hours after the bite Thereafter start rectal salines and drinks if tolerable The aim here is to increase the body fluids with a view to forestalling in some degree possible fluid loss and vaso-motor depression

(8) If the indications for amputation are present as defined above, amputate

(B) Lafer

Poisoning by *E schistosa* and *N morgani*

(1) The venoms of these snakes although absorbed quickly fix slowly and therefore the withdrawal of blood and its replacement by donor's blood may, by simple subtraction, reduce a lethal dose to a sub-lethal one

(2) When the paralyses have strongly developed and the respiration is failing, the central stimulant effect of carbon dioxide may be tried in conjunction with oxygen, both from cylinders when such are available Failing this the patient may be made to breathe in and out of a paper or rubber bag

(3) Should breathing stop, artificial respiration may be persevered with as long as the heart beats

Poisoning by *V lebetina*, *C cornutus* and *E carinatus*

The evils to be countered are syncope, hæmorrhages, cumulative toxæmia from venom constituents, necrosed tissues, uneliminated katabolic products, and bacterial toxins, destruction of blood cells, and inability to combat infection

Syncope may be fought with, the raising of the foot of the bed, the bandaging of the limbs and the use of an abdominal binder, blankets, hot-water bottles, when tolerable, half-hourly two-ounce feeds of soup, tea, coffee, or cocoa, by rectal and subcutaneous salines, by intravenous administration of Bayliss's solution, by hypodermic injections of pituitrin, adrenalin, ergotin, and digitalin or strophanthin and best of all with blood transfusion, this last gives the patient what he most stands in need of, more blood

Hæmorrhages should be countered with early and repeated calcium chloride (*vide supra*), by hæmoplastin and normal horse serum and by blood transfusions, adrenalin is also of value

The elimination of toxins presents a big problem and here again venesection and withdrawal of poisoned blood and its replacement by fresh blood or any available suitable solution would appear to be of most value If the patient's fluid content appears good, toxin elimination by all natural routes may be stimulated by diuretics, saline aperients and hot packs

Destruction of red and white blood cells can obviously only be countered with blood transfusion

Infection, notably in poisoning from *V lebetina*, must be met half way Polyvalent anti-streptococcal serum should be administered early and repeatedly Tetanus, malignant œdema, etc, will call for specific sera and in sub-acute cases an autogenous vaccine may help Locally hydrogen peroxide and Carrel-Dakin eusol treatment would seem deserving of recommendation Hot fomentations unless efficiently applied probably merely favour the growth of bacteria in sloughing areas, hot potassium permanganate baths are better Blood transfusion again appeals as

a measure of great value, meaning as it does the introduction of fresh blood with its natural content of antibodies and healthy white cells

Poisoning Doubtful

The period of doubt need not be long Weakness of the limbs, voice etc, with a strong pulse point to colubrine (*E schistosa* and *N morgani*) poisoning Marked pain and swelling at the site of injury and weak fast pulse mean a viper Examine the urine microscopically for red blood corpuscles, their presence is justification for a diagnosis of viperine poisoning if bilharziasis can be ruled out If there is great vaso-motor depression with no reaction at the site a diagnosis of bite by a non-venomous snake may be safely made and the usual restoratives coupled with assurances as to the result should be vigorously applied as in the case of patients diagnosed initially as suffering from fright and not from snake poisoning

Summary

(1) The snake population of Iraq has been discussed and five out of a total of twenty-four species noted as dangerously venomous to man These five species are a sea-snake, *Enhydrina schistosa*, a cobra, *Naja morgani*, and three vipers, *Vipera lebetina*, *Cerastes cornutus*, and *Echis carinatus*

(2) The distribution of these snakes in Iraq has been discussed and it has been claimed that *E schistosa* may be found as far upstream as Basra, and further, that *N morgani* is found throughout the country, that *V lebetina* is confined to the north-east of Iraq, taking Baghdad as the centre of the country, that *C cornutus* is fairly common on the Euphrates and west of it, but that it probably occurs rarely also throughout the country, and that *E carinatus* is confined to the Diwaniyah area of the Middle Euphrates

(3) The validity of *W ægyptia* and *N morgani* has been discussed and it has been suggested that these snakes are quite possibly conspecific and should stand as *N ægyptia*

(4) A simple key to the field identification of these venomous species has been formulated due regard having been had to the characteristics of the nineteen harmless species indigenous to the country These nineteen have been named

(5) Folk remedies for snake-bite in Iraq and snake-bite in the country in the past, remote and immediate, have been touched upon in a brief review

(6) No bites by *E schistosa* have been recorded from Iraq

(7) Two fatal bites by *N morgani* have been recorded and discussed

(8) Fifteen bites by *V lebetina* have been recorded and discussed It is shown that this snake causes most snake-bite fatalities in the country and has a lethality of 33⅓ per cent Five types of fatal result from its poisoning have been described

(9) Two probable deaths from *C cornutus* have been described

(10) Five cases of poisoning by *E carinatus* have been described, two of them ended fatally

(11) The differential diagnosis of snake-bite in Iraq has been discussed

(12) The treatment of snake-bite has been briefly discussed

(13) A scheme of treatment for snake-bite cases in Iraq has been formulated

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THE EFFECT OF INSANITARY CONDITION ON THE THYROID GLAND AND OTHER ORGANS OF THE BODY*—*concld*

BY

COLONEL R McCARRISON, CIE, KHP, IMS,
Nutritional Research Laboratories, Pasteur Institute, Coonoor, S India,

AND

K B MADHAVA, MA, AIA,
Professor of Statistics, University of Mysore

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Effect of insanitary condition on other organs.

THE presence of a 'goitre-noxa' in association with the conditions of insanitation prevailing in the present experiment having thus been conclusively demonstrated, it remains to be seen what changes are brought about by it in other organs of the body Strictly speaking we have no right to assume that because this noxa gives rise to goitre it is the cause also of the changes, now to be demonstrated, that occur in other organs It is conceivable that this noxa may be specific for the thyroid gland and that others, occurring in association with it, are responsible for such changes as are found in other organs of the body This possibility cannot, at present, be ignored Accordingly, we speak of the effect of 'insanitary condition' on other organs rather than of the effects of the 'goitre-noxa' upon them, although these effects occur coincidently with those produced on the thyroid gland itself

Statistical analysis of their weights and sizes indicate that the organs fall in three groups —

(*a*) those in which enlargement occurred the adrenals and the spleen,
(*b*) those in which diminution in size occurred the thymus and the testicles, and,
(*c*) those whose weight and size remained unchanged or were indefinite the heart, the kidneys and the liver

Significant changes in the proportionate size of any organ of the body is no more than a gross manifestation if a disturbance in balance of the normal relationships existing between different parts of the organism As such they are a relatively ready means of detecting abnormality But it is obvious that other means must be employed to determine the nature of these changes Similarly, the

* The first half of this paper has been published in the October 1932 number of the *Journal*.

non-occurrence of change, as detectable by such means, does not necessarily imply that no abnormality existed Our results are presented, therefore, as indicators of change in a later paper it is hoped to deal with the nature of this change

It is to be emphasized that the duration of the experiment was not great (110 to 120 days), more marked effects might have been observed had it been more prolonged Our results relate to the effects produced within a period of 120 days

A. The adrenal glands.—

The effect of insanitary condition on these organs is shown in Tables IV(*a*) and IV(*b*) and in Figs 7 to 10 It will be noted from them that it is exhibited as a significant increase both in their actual weights and in their size relative to that of the body, an increase, however, which is slight in comparison to that induced in the thyroid gland by the 'goitre-noxa' It will be observed [Table IV(*a*)] that at almost every range of body-weight both the actual weights of these glands and their sizes are greater in the 'insanitary' than in the 'sanitary' group Here, also, an increased degree of positive skewness of the frequency distribution diagrams is to be noted as well as the later occurrence of the modal point This effect is equally marked in the two sexes, for although the size ('r') of the adrenal glands was greater in females than in males their response to the action of insanitary condition did not differ materially in the two sexes

TABLE IV(*a*)

Showing mean adrenal-weights and mean adrenal-sizes ('r') in rats living in sanitary and in insanitary conditions (Figs 7 and 8)

Body-weight, g	Number of rats		Adrenal weight, mg		Adrenal size, mg	
	Sanitary	Insanitary	Sanitary	Insanitary	Sanitary	Insanitary
60—69		1		20		31
70—79	1	1	21	15	27	20
80—89		2		26		30
90—99	4	3	39	41	40	43
100—109	16	7	29	30	27	24
110—119	26	20	32	37	26	32
120—129	14	20	35	37	29	30
130—139	4	15	35	44	26	33
140—149	12	10	29	39	20	28
150—159	18	8	25	29	16	26
160—169	16	14	30	33	18	21
170—179	16	12	28	36	16	20
180—189	2	13	28	34	16	18
190—199		5		31		16
200—209		1		25		12
210—219						
220—229		1		65		24

TABLE IV(*b*)

Showing frequency distributions of adrenal-weights and of adrenal-sizes ('r') in rats living in sanitary and in insanitary conditions (Figs 9 and 10)

Range of adrenal weight, mg	NUMBER OF RATS		Range of adrenal size, mg	NUMBER OF RATS	
	Sanitary	Insanitary		Sanitary	Insanitary
10—14 9		1	0—9	1	3
15—19 9	6	7	10—19	51	31
20—24 9	23	11	20—29	48	50
25—29 9	33	14	30—39	26	36
30—34 9	33	19	40—49	3	13
35—39 9	21	25			
40—44 9	8	23			
45—49 9	4	20			
50—54 9	1	6			
55—59 9		1			
60—64 9		4			
65—69 9		2			
TOTALS	129	133		129	133
Mean and standard error	30 8 ± 0 63	37 6 ± 0 97		23 4 ± 0 70	26 9 ± 0 80
Standard deviation (σ)	7 1	11 0		8 3	9 8
Co efficient of variation	23	29		36	36
Difference between means	6 8 ± 1 16			3 51 ± 1 13	
Significance	t=5 89, significant			t=3 20, significant	

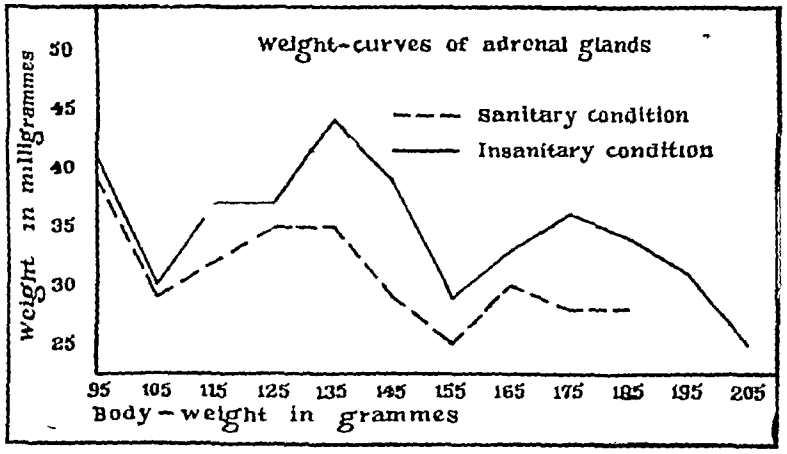

Fig 7 Weights of the adrenal glands at different body-weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the higher adrenal weights at all body-weights

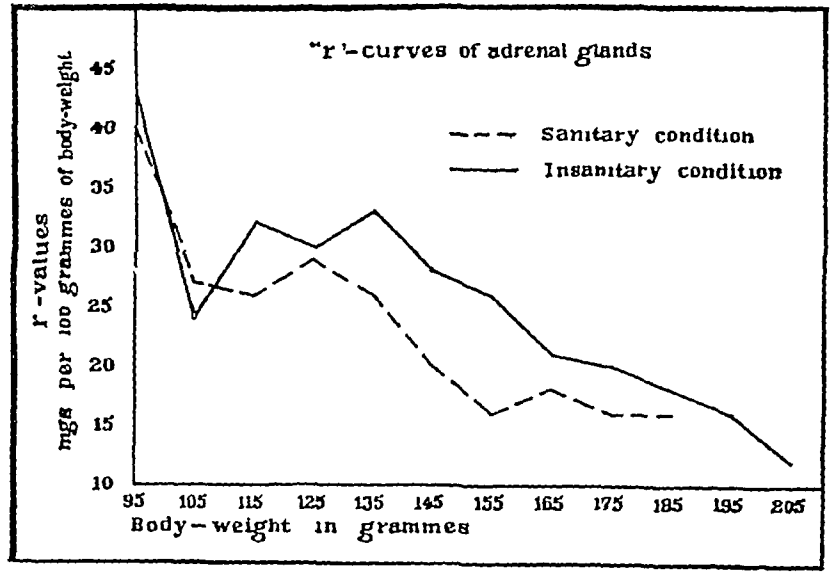

Fig 8 Size ('r') of the adrenal glands at different body-weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the higher adrenal-sizes at all body-weights over 110 grammes

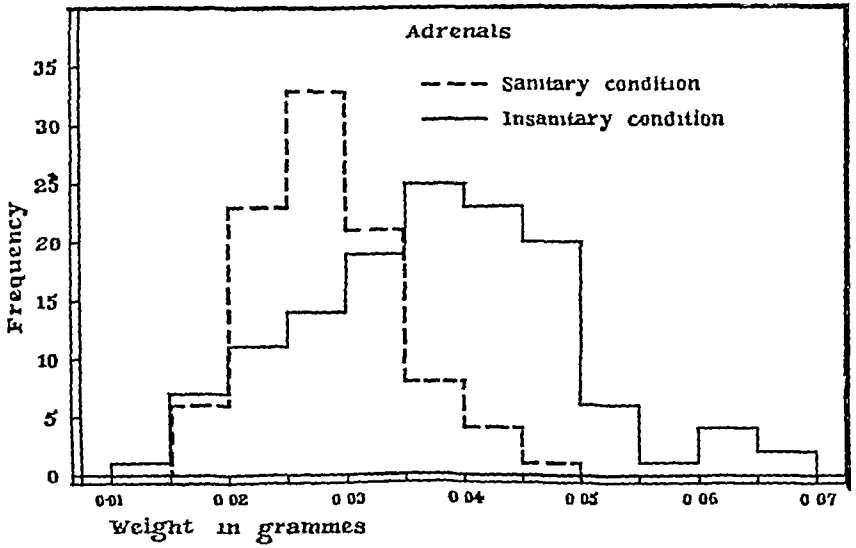

Fig 9 Frequency distributions of adrenal weights in the two groups Note the positive skewness and the later occurrence of the modal point in the insanitary group

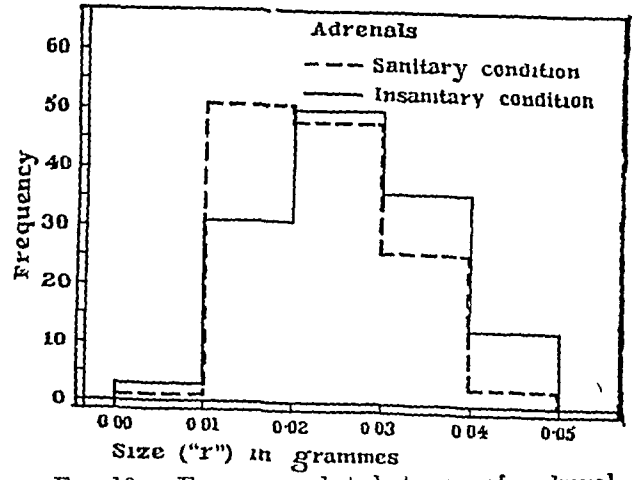

Fig 10 Frequency distributions of adrenal-sizes ('r') in the two groups Note same features as in Fig 9

B The spleen.

The effect of insanitary condition on this organ is shown in Tables V(*a*) and V(*b*) and in Figs 11 to 14 Here also it is one of significant increase both in the actual and in the relative weight (' r ') of the organ The observation recorded in another place is thus confirmed (McCarrison and Madhava, 1932) Attention is directed to the increased weight and size of the spleen at almost every range of body-weight, as well as to the positive skewness and later occurrence of the modal point in the frequency distribution diagrams of the insanitary group This effect is equally marked in the two sexes

TABLE V(*a*)

Showing mean spleen-weights and mean spleen-sizes (' r ') in rats living in sanitary and in insanitary conditions (Figs 11 and 12)

Body weight, g	NUMBER OF RATS		SPLEEN WEIGHT, G		SPLEEN SIZE, G	
	Sanitary	Insanitary	Sanitary	Insanitary	Sanitary	Insanitary
60—69		1		0 28		0 40
70—79	1	1	0 45	0 38	0 57	0 50
80—89		2		0 52		0 57
90—99	4	3	0 41	0 40	0 42	0 43
100—109	16	7	0 43	0 42	0 40	0 48
110—119	26	20	0 44	0 49	0 38	0 43
120—129	14	20	0 49	0 51	0 40	0 41
130—139	4	15	0 44	0 53	0 33	0 40
140—149	12	10	0 49	0 54	0 34	0 37
150—159	18	8	0 49	0 57	0 32	0 38
160—169	16	14	0 56	0 58	0 35	0 36
170—179	16	12	0 58	0 66	0 33	0 38
180—189	2	13	0 46	0 62	0 27	0 34
190—199		5		0 72		0 37
200—209		1		0 77		0 38
210—219						
220—229		1		1 09		0 48

TABLE V(*b*)

Showing frequency distributions of spleen-weights and of spleen-sizes ('r') in rats living in sanitary and in insanitary conditions (Figs 13 and 14)

Range of spleen weight, g	Number of rats		Range of spleen size, g	Number of rats	
	Sanitary	Insanitary		Sanitary	Insanitary
			0 10—0 19	2	
0 20—0 29	4	2	0 20—0 29	30	7
0 30—0 39	35	10	0 30—0 39	56	66
0 40—0 49	31	32	0 40—0 49	29	48
0 50—0 59	31	48	0 50—0 59	10	11
0 60—0 69	21	28	0 60—0 69	2	1
0 70—0 79	4	9			
0 80—0 89	3	2			
0 90—0 99		1			
1 00—1 09		1			
Totals	129	133		129	133
Mean and standard error	0 4919 ± 0 1118	0 5523 ± 0 0108		0 3663 ± 0 0086	0 3996 ± 0 0065
Standard deviation (σ)	0 1330	0 1249		0 0971	0 0752
Coefficient of variation	27	23		27	19
Difference between means	0 0604 ± 0 0160			0 0333 ± 0 0107	
Significance	t=3 8, significant			t=3 2, significant	

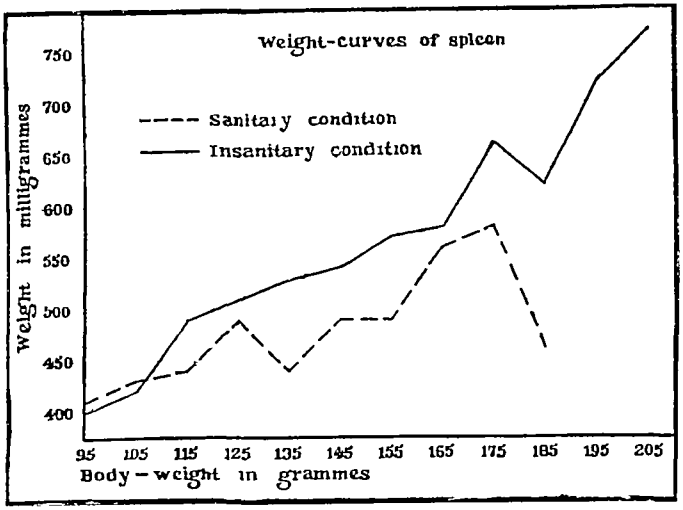

Fig 11 Weight of the spleen at different body weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the greater weight of the organ at all body weights over 110 grammes

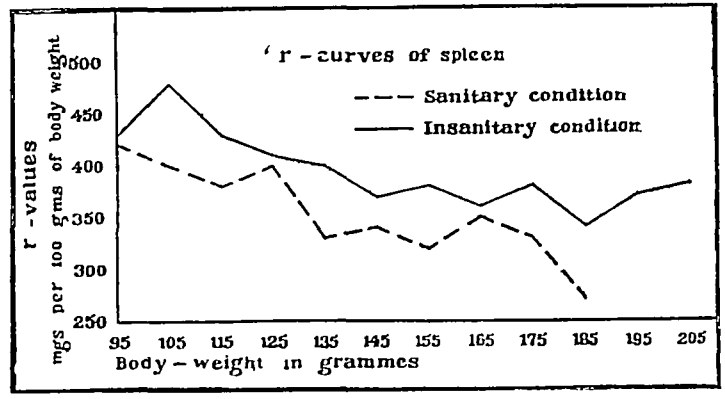

Fig 12 Size ('r') of the spleen at different body weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the uniformly longer size of the organ in the insanitary group

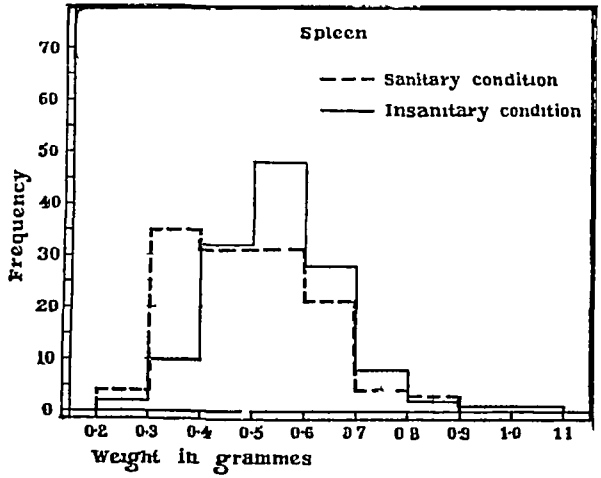

Fig 13 Frequency distributions of spleen weights in the two groups Note the later occurrence of the modal point in the insanitary group

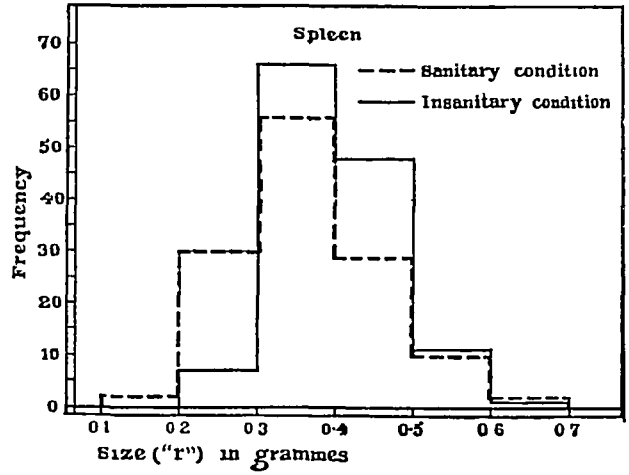

Fig 14 Frequency distributions of spleen sizes ('r') in the two groups Note same features as in Fig 13

C. The testicles.

In contrast to the enlargement of the thyroid, adrenal glands and spleen, the testes show an effect of the opposite order These organs are significantly reduced in size , the magnitude of this reduction being, however, less great than that of the increase in size of the adrenals and spleen The interest of this observation is considerable, having regard to the condition of the sex-organs in endemic cretinism

Attention is here directed to the *negative* skewness of the frequency distribution diagrams and to the *earlier* occurrence of the modal point in the insanitary group

These results are shown in Tables VI(*a*) and VI(*b*) and in Figs 15 to 18 —

TABLE VI(*a*)

Showing mean testicle-weights and mean testicle-sizes ('1') in rats living in sanitary and in insanitary conditions (Figs 15 and 16)

Body weight, g	Number of rats		Testicle weight, g		Testicle size, c	
	Sanitary	Insanitary	Sanitary	Insanitary	Sanitary	Insanitary
60—69		1		1 15		1 77
70—79						
80—89						
90—99		3		1 51		1 60
100—109	1		2 42		2 26	
110—119	1	1	2 85	2 14	2 48	1 89
120—129		1		2 40		1 86
130—139	2	2	1 96	1 83	1 48	1 37
140—149	12	3	2 19	1 93	1 52	1 34
150—159	17	6	2 22	1 98	1 45	1 31
160—169	16	14	2 21	2 21	1 35	1 34
170—179	16	12	2 35	2 24	1 34	1 27
180—189	2	13	2 41	2 31	1 32	1 26
190—199		5		2 13		1 10
200—209		1		2 28		1 11
210—219						
220—229		1		2 59		1 14

TABLE VI(*b*)

Showing frequency distributions of testicle-weights and of testicle-sizes ('r') in rats living in sanitary and in insanitary conditions (Figs 17 and 18)

Range of testicle weight, g	NUMBER OF RATS		Range of testicle size, g	NUMBER OF RATS	
	Sanitary	Insanitary		Sanitary	Insanitary
0 90—1 09		1	1 00—1 09	1	3
1 10—1 29		1	1 10—1 19	7	12
1 30—1 49			1 20—1 29	9	16
1 50—1 69	1	2	1 30—1 39	10	13
1 70—1 89	4	6	1 40—1 49	15	8
1 90—2 09	10	11	1 50—1 59	13	5
2 10—2 29	15	19	1 60—1 69	4	1
2 30—2 49	22	15	1 70—1 79	2	1
2 50—2 69	10	4	1 80—1 89	1	2
2 70—2 89	1	3	1 90—1 99		
2 90—3 09	2		2 00—2 09	1	1
			2 10—2 19		
			2 20—2 29	1	
			2 30—2 39		
			2 40—2 49	1	
TOTALS	65	62		65	62
Mean and standard error	2 32 ± 0 03	2 17 ± 0 04		1 45 ± 0 03	1 34 ± 0 025
Standard deviation (σ)	0 27	0 33		0 244	0 198
Co efficient of variation	12	15		17	15
Difference between means	0 1427 ± 0 0548			0 1144 ± 0 0395	
Significance	t = 2 6, significant			t = 2 9, significant	

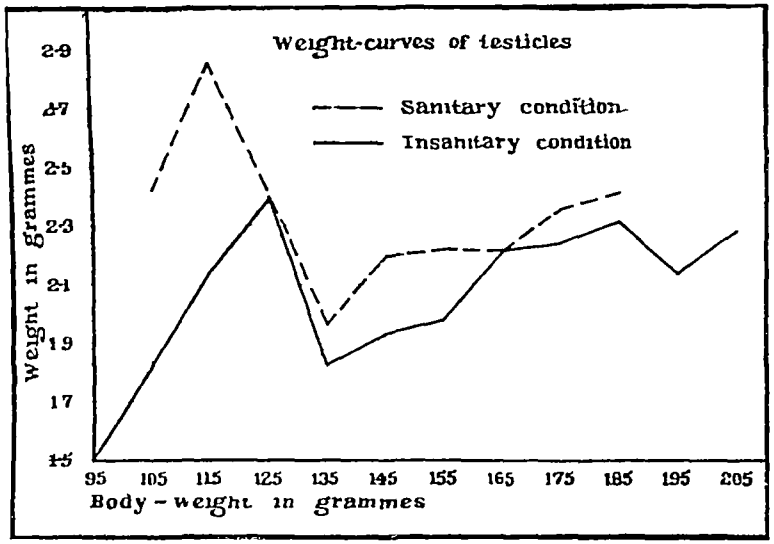

Fig 15 Weight of the testicles at different body-weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the smaller weight of these organs at some ranges of body-weight

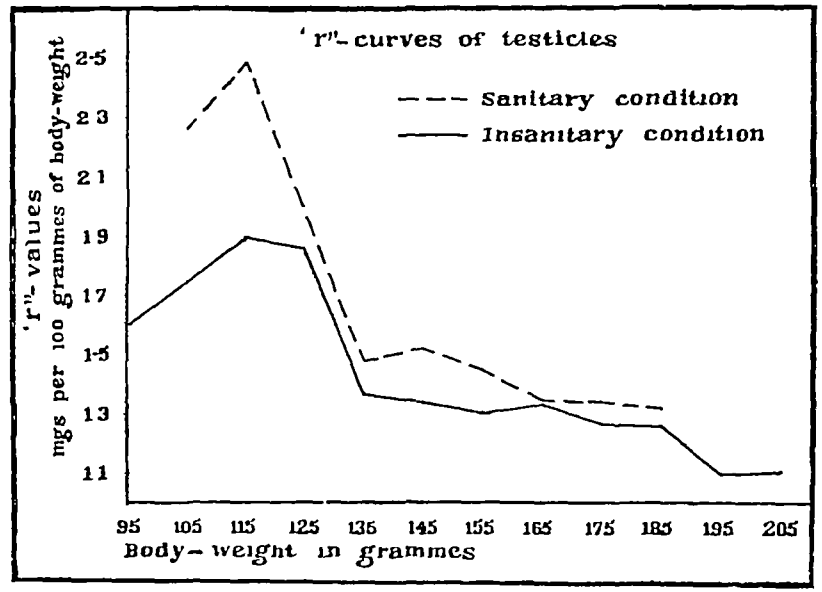

Fig 16 Size ('r') of the testicles at different body weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the smaller size of these organs at all body-weights

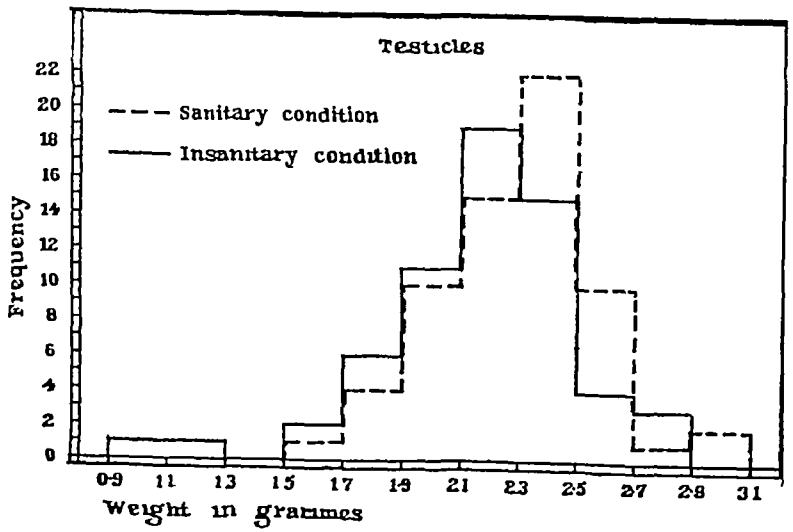

Fig 17 Frequency distributions of testicle weights in the two groups Note the negative skewness and the earlier occurrence of the modal point in the insanitary group

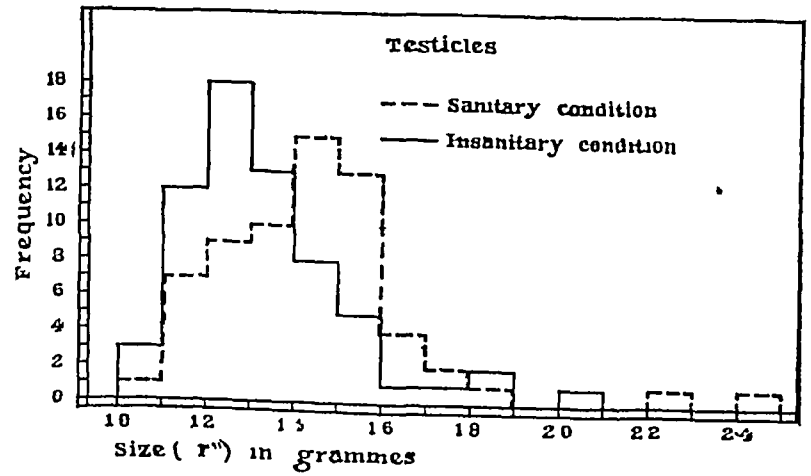

Fig 18 Frequency distributions of testicle sizes (r ') in the two groups Note same features as in Fig 17

D The thymus

Here also the effect of insanitary condition was to cause a significant decrease in both the actual weight and size of the thymus This diminution is more marked than in the testes It will be noted [Table VII(*a*)] that at every range of body-weight both the thymus-weight and the thymus-size are less in the 'insanitary' than in the 'sanitary' group The frequency distribution diagrams also show the features associated with diminution in size negative skewness and earlier appearance of the modal point This effect of insanitary condition was more marked in males than in females (Table XI)

TABLE VII(*a*)

Showing mean thymus-weights and mean thymus-sizes ('i') in rats living in sanitary and in insanitary conditions (Figs 19 and 20)

Body weight, g	NUMBER OF RATS		THYMUS WEIGHT, G		THYMUS SIZE, G	
	Sanitary	Insanitary	Sanitary	Insanitary	Sanitary	Insanitary
60—69		1		0 090		0 138
70—79	1	1	0 080	0 080	0 101	0 105
80—89		1		0 040		0 046
90—99	4	3	0 120	0 060	0 118	0 064
100—109	16	7	0 120	0 096	0 111	0 067
110—119	26	20	0 130	0 109	0 099	0 091
120—129	14	20	0 120	0 112	0 097	0 090
130—139	4	15	0 090	0 121	0 068	0 090
140—149	12	10	0 140	0 117	0 096	0 081
150—159	18	8	0 120	0 111	0 077	0 073
160—169	16	14	0 140	0 113	0 071	0 069
170—179	16	12	0 110	0 108	0 064	0 062
180—189	2	13	0 120	0 095	0 063	0 051
190—199		5		0 106		0 054
200—209		1		0 095		0 046
210—219						
220—229		1		0 150		0 067

TABLE VII(*b*)

Showing frequency distributions of thymus-weights and of thymus-sizes ('i') in rats living in sanitary and in insanitary conditions (Figs 21 and 22)

Range of thymus weight, mg	NUMBER OF RATS		Range of thymus size, mg	NUMBER OF RATS	
	Sanitary	Insanitary		Sanitary	Insanitary
40—49		1	0—9	1	
50—59		9	10—19	1	
60—69	3	3	20—29		2
70—79	3	3	30—39	1	7
80—89	16	12	40—49	5	10
90—99	15	16	50—59	12	15
100—109	11	15	60—69	13	19
110—119	13	16	70—79	22	15
120—129	17	14	80—89	13	19
130—139	10	17	90—99	11	13
140—149	6	6	100—109	10	11
150—159	10	9	110—119	14	6
160—169	12	3	120—129	9	6
170—179	5	3	130—139	7	4
180—189	3	1	140—149	3	1
190—199	5		150—159	2	
			160—169	2	
			170—179		
			180—189	2	
			230—239	1	
TOTALS	129	128		129	128
Mean and standard error	125 1 ± 2 9	112 7 ± 2 7		92 8 ± 3 0	78 6 ± 2 4
Standard deviation (σ)	33 2	30 5		34 2	26 8
Coefficient of variation	27	27		37	34
Difference between means	12 4 ± 4 0			14 2 ± 3 85	
Significance	t=3 0, significant			t=3 7, significant	

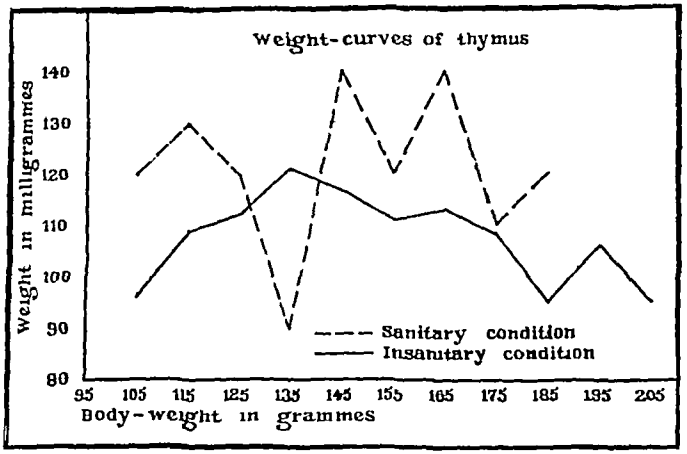

Fig 19 Weight of the thymus at different body weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the smaller weights at all body weights except 135 grammes at this body weight there were only 4 rats in the sanitary, as compared with 15 in the insanitary, group Table VII(*a*)

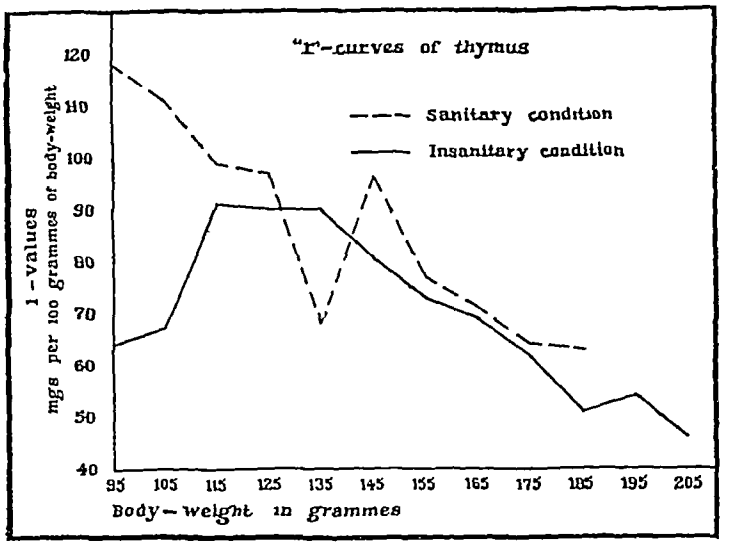

Fig 20 Size ('r') of the thymus at different body weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the smaller size of the organ at all body weights except 135 grammes

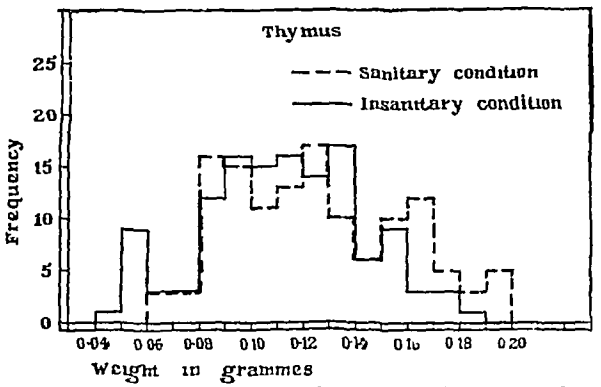

Fig 21 Frequency distributions of thymus weights in the two groups Note shift of the diagram to the left in the insanitary group

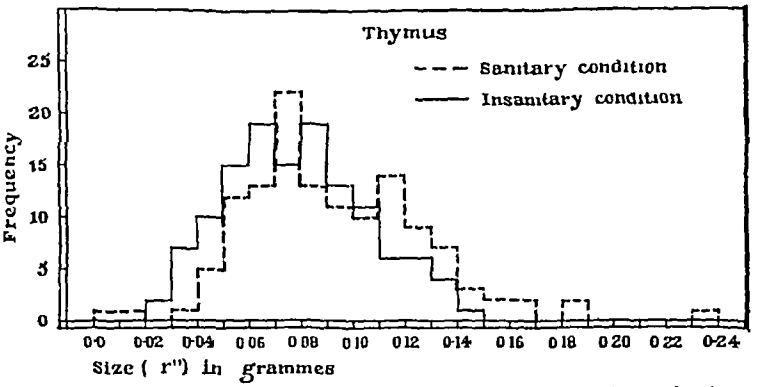

Fig 22 Frequency distributions of thymus sizes ('r') in the two groups Note shift of the diagram to the left in the insanitary group

E. The liver.

The effect of insanitary condition on this organ is less definite than that observed in any other organ For although there is a significant difference in the liver-sizes (' r ') yet this difference is not reproduced in the actual weight of the organ in the two groups The first finding must, therefore, be mainly an effect of body-weight In females the liver was smaller than in males, ranging in weight from 7 5 to 8 0 g for a body-weight of about 118 g , while in males it weighed about 10 g for a body-weight of 160 g Nevertheless, it will be noted from the frequency distribution diagrams (Figs 25 and 26) that the modal point occurs earlier in the insanitary than in the sanitary group

Attention was drawn in a previous publication (McCarrison and Madhava, 1932) to the increase in size of the liver which appeared to result from insanitary condition The present results are in opposition to this observation But the dietary conditions were not the same in the two cases and possibly it is to this that the disparity is due For the present, therefore, the effects of insanitary condition *per se* on the liver must be regarded as indefinite

TABLE VIII(*a*)

Showing mean liver-weights and mean liver-sizes (' r ') in rats living in sanitary and in insanitary conditions (Figs 23 and 24)

Body weight, g	NUMBER OF RATS		LIVER WEIGHT, G		LIVER SIZE, G	
	Sanitary	Insanitary	Sanitary	Insanitary	Sanitary	Insanitary
60—69		1		4 10		6 31
70—79	1	1	5 6	5 16	7 20	6 47
80—89		2		4 86		5 68
90—99	4	3	6 6	5 52	6 80	5 87
100—109	16	7	6 7	6 43	6 35	5 88
110—119	26	20	7 6	6 91	6 63	6 00
120—129	14	20	7 6	7 44	6 14	5 94
130—139	4	15	7 6	7 71	5 72	5 83
140—149	12	10	9 2	8 69	6 40	6 02
150—159	18	8	9 8	9 17	6 39	6 01
160—169	16	14	10 3	10 06	6 26	6 11
170—179	16	12	11 2	10 33	6 45	5 85
180—189	2	13	11 2	11 39	6 15	6 19
190—199		5		12 27		6 31
200—209		1		11 78		5 75
210—219						
220—229		1		13 40		5 88

TABLE VIII(*b*)

Showing frequency distributions of liver-weights and of liver-sizes ('r') in rats living in sanitary and in insanitary conditions (Figs 25 and 26)

Range of liver weight, g	Number of rats		Range of liver size, g	Number of rats	
	Sanitary	Insanitary		Sanitary	Insanitary
4 0—4 9		2	4 0—4 9	4	3
5 0—5 9	6	8	5 0—5 9	26	64
6 0—6 9	23	21	6 0—6 9	78	62
7 0—7 9	24	30	7 0—7 9	16	3
8 0—8 9	16	18	8 0—8 9	4	1
9 0—9 9	18	16	9 0—9 9	1	
10 0—10 9	28	19			
11 0—11 9	11	13			
12 0—12 9	3	2			
13 0—13 9		4			
Totals	129	133		129	133
Mean and standard error	8 66 ± 0 16	8 63 ± 0 18		6 45 ± 0 07	6 01 ± 0 05
Standard deviation (σ)	1 85	2 05		0 80	0 62
Co efficient of variation	21	24		12	10
Difference between means	0 03 ± 0 24			0 43 ± 0 09	
Significance	t = 0 1, not significant			t = 4 9, significant	

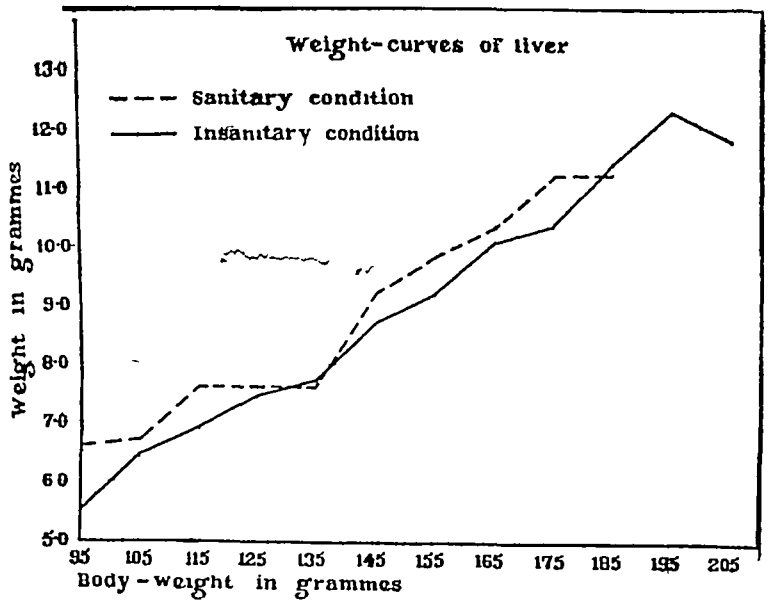

Fig 23 Weight of the liver at different body-weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the tendency (not, however, statistically significant) to diminution in weight of the liver in the insanitary group

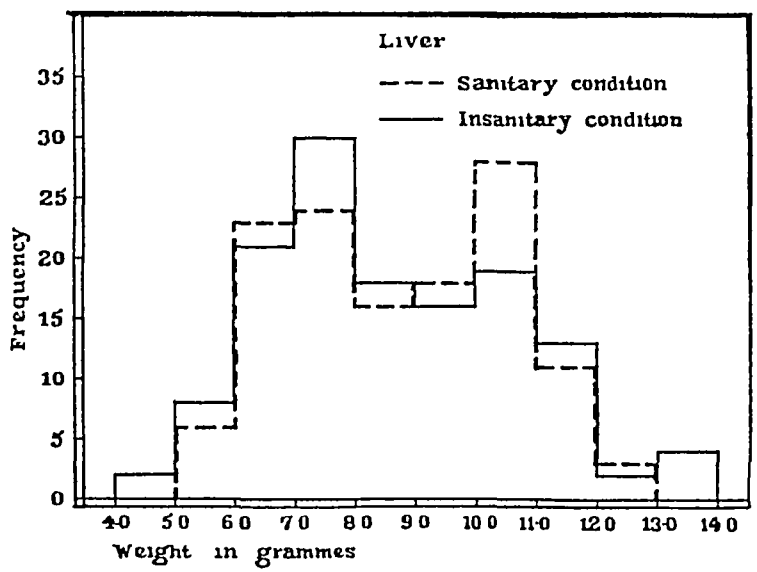

Fig 25 Frequency distributions of liver weights in the two groups

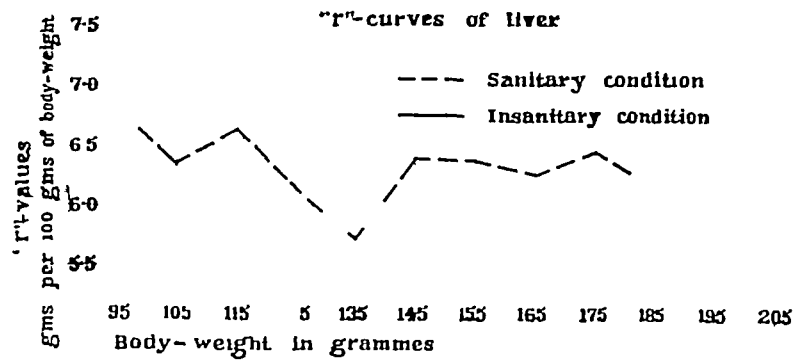

Fig 24 Size ('r') of the liver at different body-weights in rats living in sanitary and in insanitary conditions while being fed on the same diet Note the smaller size of the organ at all body weights except 135 grammes at which weight there were only 4 rats in the sanitary group (*See* text)

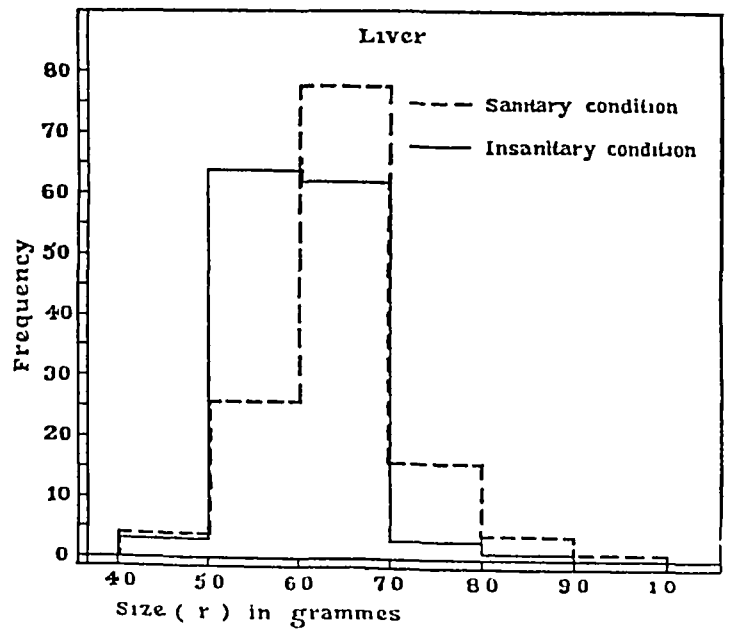

Fig 26 Frequency distributions of liver-sizes ('r') in the two groups Note earlier occurrence of the modal point in the insanitary group

F. The kidneys

Insanitary condition had no effect on these organs within the period of the experiment, so far as could be determined by statistical methods of observation

TABLE IX(*a*)

Showing mean kidney-weights and mean kidney-sizes ('r') in rats living in sanitary and in insanitary conditions (Figs 27 and 28)

Body weight, g	NUMBER OF RATS		KIDNEY WEIGHT, G		KIDNEY SIZE, G	
	Sanitary	Insanitary	Sanitary	Insanitary	Sanitary	Insanitary
60—69		1		1 01		1 55
70—79	1	1	0 85	0 95	1 08	1 25
80—89		2		1 47		1 72
90—99	2	3	1 20	1 08	1 22	1 15
100—109	16	7	1 36	1 15	1 35	0 97
110—119	26	20	1 27	1 28	0 99	1 11
120—129	14	20	1 28	1 36	1 05	1 08
130—139	4	15	1 12	1 33	0 84	1 00
140—149	12	10	1 46	1 49	1 01	1 03
150—159	18	8	1 58	1 53	1 03	1 01
160—169	16	14	1 55	1 57	0 95	0 95
170—179	16	12	1 64	1 67	0 94	0 95
180—189	2	13	1 84	1 70	1 02	0 95
190—199		5		1 85		0 95
200—209		1		1 85		0 90
210—219						
220—229		1		2 21		0 97

TABLE IX(*b*)

Showing frequency distributions of kidney-weights and of kidney-sizes ('i') in rats living in sanitary and in insanitary conditions (Figs 29 and 30)

Range of kidney weight, g	NUMBER OF RATS		Range of kidney size, g	NUMBER OF RATS	
	Sanitary	Insanitary		Sanitary	Insanitary
0 80—0 89	1		0 60—0 69		3
0 90—0 99	4	4	0 70—0 79	5	2
1 00—1 09	7	10	0 80—0 89	22	19
1 10—1 19	9	11	0 90—0 99	21	37
1 20—1 29	22	16	1 00—1 09	36	33
1 30—1 39	17	21	1 10—1 19	18	19
1 40—1 49	18	11	1 20—1 29	12	11
1 50—1 59	10	14	1 30—1 39	7	2
1 60—1 69	19	19	1 40—1 49	3	1
1 70—1 79	13	5	1 50—1 59	1	4
1 80—1 89	3	10	1 60—1 69	1	1
1 90—1 99	4	7	1 70—1 79	1	
2 00—2 09		2	1 80—1 89		
2 10—2 19		2	1 90—1 99		1
2 20—2 29		1			
TOTALS	127	133		127	133
Mean and standard-error	1 44 ± 0 02	1 47 ± 0 03		1 06 ± 0 0165	1 04 ± 0 0168
Standard deviation (σ)	0 2469	0 2929		0 1861	0 1925
Co efficient of variation	17	20		17 6	18 5
Difference between means	0 0337 ± 0 0344			0 0155 ± 0 0235	
Significance	t=0 9, not significant			t=0 7, not significant	

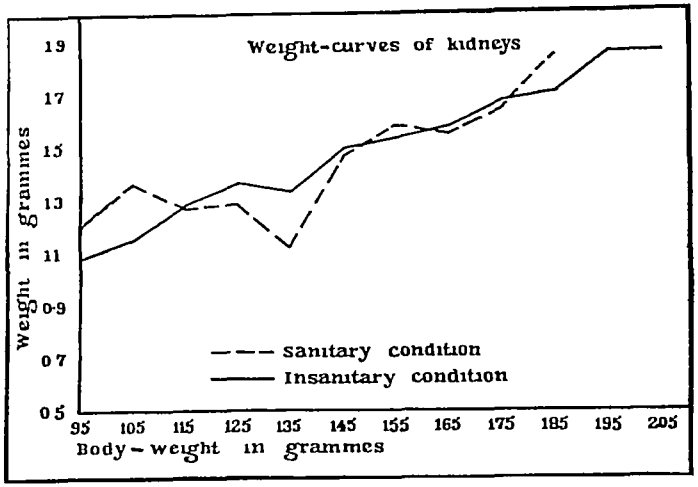

Fig 27 Weight of the kidneys at different body weights in rats living in sanitary and in insanitary conditions while being fed on the same diet

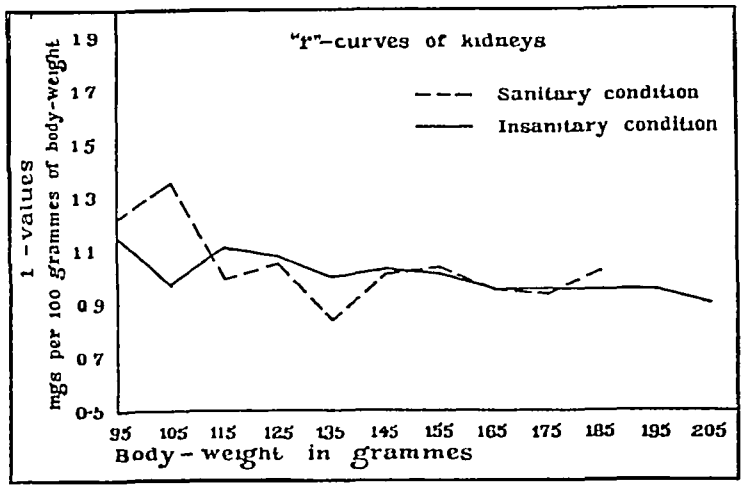

Fig 28 Size ('r') of the kidneys at different body weights in rats living in sanitary and in insanitary conditions while being fed on the same diet

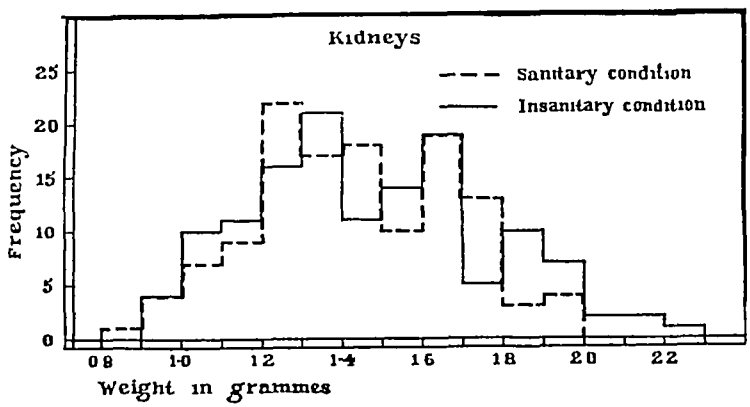

Fig 29 Frequency distributions of kidney weights in the two groups The two distributions do not differ appreciably

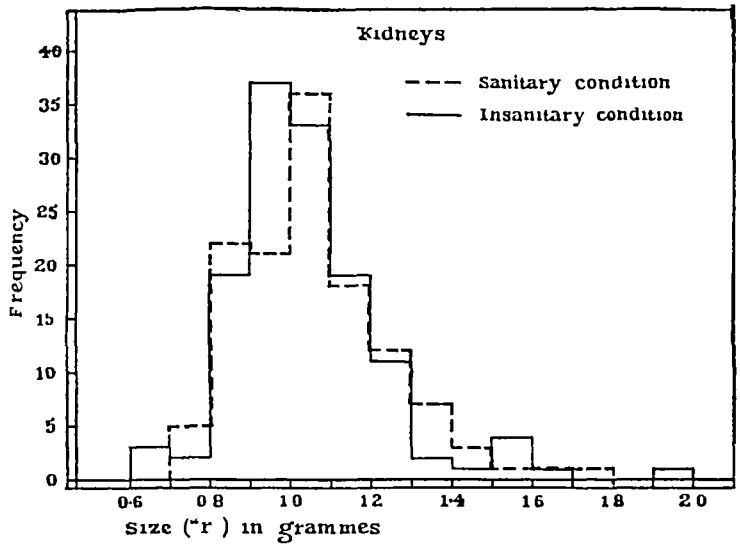

Fig 30 Frequency distributions of kidney sizes ('r') in the two groups The two distributions do not differ appreciably

G. The heart.

The heart also showed no significant change either in weight or size consequent on exposure of the animals to the insanitary condition for a period of 120 days

TABLE X(*a*)

Showing mean heart-weights and mean heart-sizes ('r') in rats living in sanitary and in insanitary conditions (Figs 31 and 32)

Body-weight, g	Number of rats		Heart-weight, g		Heart size, g	
	Sanitary	Insanitary	Sanitary	Insanitary	Sanitary	Insanitary
60—69		1		0 49		0 75
70—79	1	1	0 57	0 35	0 72	0 46
80—89		1		0 38		0 44
90—99	4	3	0 58	0 45	0 60	0 48
100—109	16	7	0 59	0 50	0 56	0 40
110—119	26	20	0 57	0 58	0 50	0 50
120—129	14	20	0 63	0 63	0 51	0 50
130—139	4	15	0 64	0 65	0 48	0 49
140—149	12	10	0 63	0 63	0 44	0 44
150—159	18	8	0 65	0 65	0 42	0 43
160—169	16	14	0 69	0 67	0 42	0 41
170—179	16	12	0 71	0 68	0 41	0 39
180—189	2	13	0 69	0 75	0 38	0 41
190—199		5		0 77		0 39
200—209		1		0 71		0 34
210—219						
220—229		1		0 72		0 40

TABLE X(*b*)

Showing frequency distributions of heart-weights and of heart-sizes ('r') in rats living in sanitary and in insanitary conditions (Figs 33 and 34)

Range of heart weight, g	NUMBER OF RATS		Range of heart-size, g	NUMBER OF RATS	
	Sanitary	Insanitary		Sanitary	Insanitary
			0 20—0 29	1	
0 30—0 39		2	0 30—0 39	16	24
0 40—0 49	9	9	0 40—0 49	72	73
0 50—0 59	37	24	0 50—0 59	30	33
0 60—0 69	55	61	0 60—0 69	7	1
0 70—0 79	26	30	0 70—0 79	3	1
0 80—0 89	2	5			
0 90—0 99		1			
TOTALS	129	132		129	132
Mean and standard error	0 63 ± 0 08	0 65 ± 0 09		0 48 ± 0 008	0 46 ± 0 006
Standard deviation (σ)	0 089	0 102		0 086	0 072
Co efficient of variation	14	16		18	15
Difference between means	0 0156 ± 0 0119			0 0165 ± 0 0099	
Significance	t = 1 3, not significant			t = 1 67, not significant	

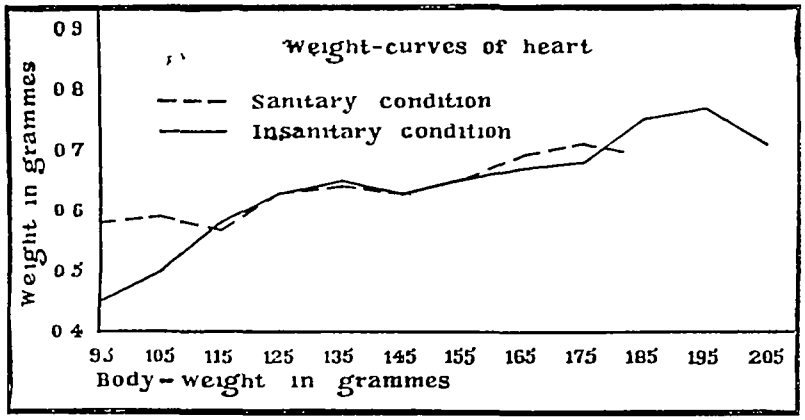

Fig 31 Weights of the heart at different body-weights in rats living in sanitary and in insanitary conditions while being fed on the same diet The weight curves in the two groups are practically identical at all body-weights above 115 grammes

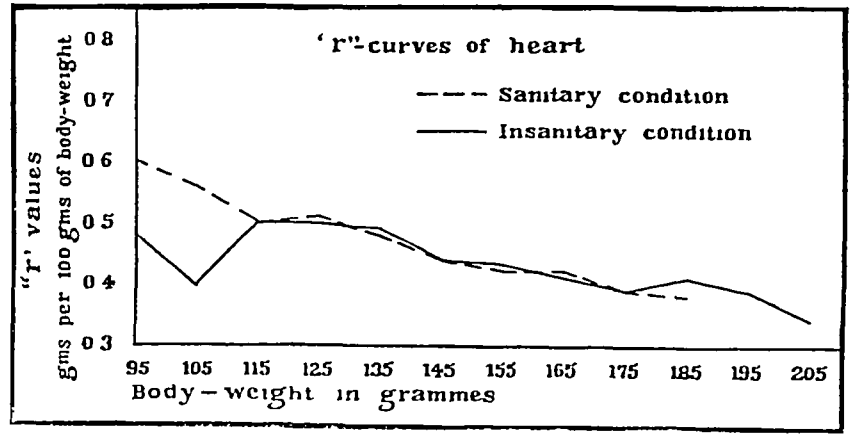

Fig 32 Size ('r') of the heart at different body weights in rats living in sanitary and in insanitary conditions The 'r'-curves in the two groups are practically identical at all body-weights above 115 grammes

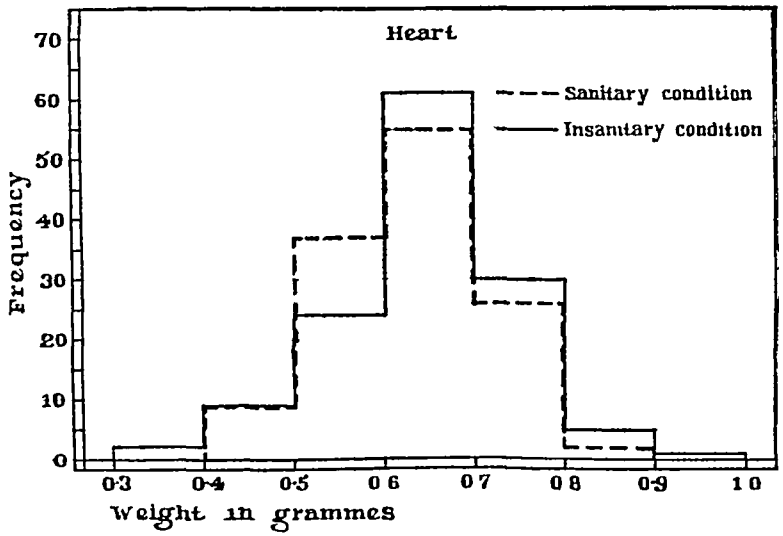

Fig 33 Frequency distributions of heart-weights in the two groups The distributions are practically identical

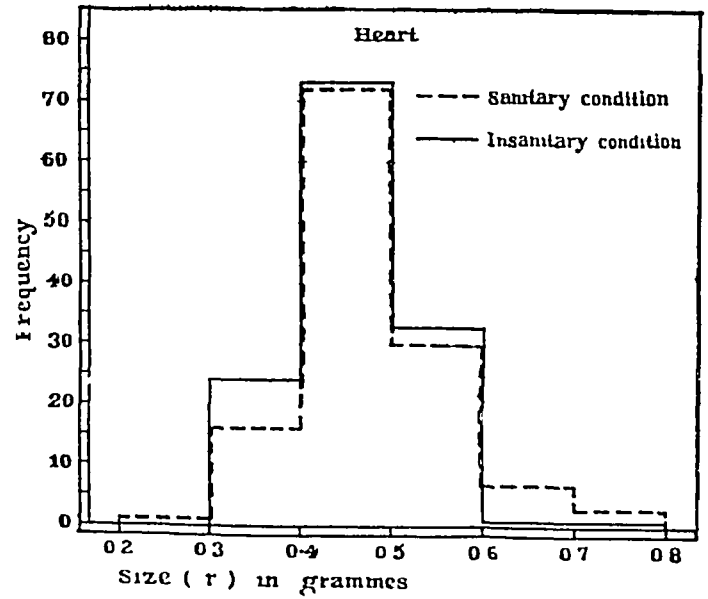

Fig 34 Frequency distributions of heart-sizes ('r') in the two groups The distributions are practically identical

Effect of insanitary condition, distinguished by sex

The mean organ-weights and organ-sizes—the latter calculated from aggregate figures and, therefore, approximate—are set out in Table XI which is self-explanatory —

TABLE XI

Showing mean weight and size ('r') of organs, distinguished by sex

	MEAN WEIGHT				MEAN SIZE (APPROX.)			
	SANITARY		INSANITARY		SANITARY		INSANITARY	
	Males	Females	Males	Females	Males	Females	Males	Females
Body weight, g	166	123	158	114				
Thyroid, mg	26 3	18 6	8 5	7 9	15 8	15 1	5 3	6 9
Heart, g	0 68	0 60	0 68	0 58	0 41	0 49	0 41	0 51
Liver, g	10 05	7 29	10 11	8 00	6 04	5 93	6 40	7 02
Spleen, g	0 60	0 51	0 52	0 43	0 36	0 41	0 33	0 38
Thymus g	0 100	0 116	0 124	0 118	0 06	0 09	0 08	0 10
Kidneys, g	1 60	1 33	1 56	1 24	0 96	1 07	0 99	1 09
Adrenals, g	0 0340	0 0385	0 0279	0 0324	0 021	0 031	0 018	0 029
Testes, g	2 17		2 32		1 34		1 45	

Effect of goitre on the heart and other organs

The size ('r') of the thyroid gland of rats living in sanitary conditions lay between 3 and 11 9 mg per 100 g of body-weight, and between 6 and 27 9 mg in those living in insanitary conditions The actual frequency distributions in both groups are set out in Table III(*b*) (Fig 6) Reference to these frequency distributions will show that 110 out of the 133 animals in the 'insanitary' group had thyroid glands whose sizes ('r') exceeded the maximum size (11 9) in the 'sanitary' group These may be arranged in the ten subdivisions, set out below, according to the increasing size of the organ The subdivisions are suggested by the mean value and the standard deviation of 'r' in the 'sanitary' group Our previous work (McCarrison and Madhava, 1932) has led us to postulate that values in excess

Summary of results.

(1) The goitrogenic potency of cabbage varies with season It appears to be related in some way to rainfall, being at its maximum during and after the heavy autumn rains, in this locality, and at its minimum during the dry months of the year

(2) Albino rats fed on a diet of cabbage, whole wheat and *cholam* (*Andropagan sorghum*)—which was not in itself goitrogenic—became goitrous when living under conditions of gross insanitation, but remained goitre-free when living under conditions of scrupulous cleanliness

(3) The existence of a 'goitre-noxa', of unknown nature, in association with insanitary condition has been demonstrated

(4) The greater the degree of insanitation the larger was the size of the goitres produced by it

(5) The size of the goitres, produced in insanitary conditions, increased with increasing body-weight (and age)

(6) The effect of insanitary condition is not limited to enlargement of the thyroid gland Associated with the hypertrophy of this organ there is an increase in size of the adrenal glands and spleen and a reduction in size of the testicles and thymus, the heart and kidneys remained unaffected in point of size, while the effect on the liver was indefinite, within the period of the experiment (120 days) The pituitary body, pancreas and brain were not examined

(7) The very evident goitre produced by gross insanitation in albino rats, fed on a diet of cabbage, whole wheat and *cholam*, had, within the period of this experiment, no correlated effect on the heart nor on any of the other organs examined

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Ind Jour Med Res, XX, 3, *January*, 1933

EFFECT OF AN EXCLUSIVE DIET OF CABBAGE ON THE INTERNAL ORGANS OF RABBITS

BY

R McCARRISON AND G SANKARAN,

Nutritional Research Laboratories, I R F A, Coonoor,

WITH

A STATISTICAL EXAMINATION OF THE DATA

BY

K B MADHAVA,

University of Mysore

[Received for publication, August 1, 1932]

IN previous communications from these Laboratories (McCarrison, 1931, McCarrison and Madhava, 1932, 1932*a*) an account was given of the goitre-producing action of cabbage grown in the Nilgiri Hills It was shown that its potency in this regard varies with season and appears to be related to rainfall, being greater during and after the monsoon rains and slight or nil during the dry season

Purpose of the investigation.

The object of the investigation, with which this paper deals, was three-fold to produce large, easily palpable, goitres in rabbits by feeding them for long periods on an exclusive diet of fresh, raw cabbage, to learn whether the enlargement of the thyroid gland so induced, was associated with any significant change in size of other organs of the body, and to ascertain whether any such change was accompanied with alterations in the H ion activity of organs exhibiting it

The work of Marine *et al* (1929), as well as our own (1931, 1932), had seemed to indicate that the longer the animals were fed on the cabbage diet the larger the resultant goitres would be But the matter did not prove to be so simple as this, for seasonal variations in the goitrogenic potency of cabbage came into play and were associated with corresponding variations—rise or fall as the case might be—in the size of the thyroid gland (McCarrison and Madhava, 1932*a*) In the present investigation a period—October to December 1931—of heavy rainfall and of high

goitrogenic potency of cabbage was followed by one—January to April 1932—of very scanty rainfall and low goitrogenic potency of cabbage, the investigation ending during the latter period

Experiments confined to these two periods showed that in the former the goitrogenic potency of cabbage was such that it caused an increase in size (' r ') of the thyroid gland at the rate of approximately 10 mg per day, an experiment lasting 56 days during this period resulted in the size of the gland being, on the average, well over 700 mg per kilogram of body-weight On the other hand, a similar experiment confined to the second period resulted in the size of the gland averaging little more than 100 mg Both these periods were included in the present experiment, on its termination the average size of the thyroid was found to be 252 mg Presumably, therefore, a precipitate fall in the size of the organ occurred from December, when it was at its highest level, to April a process of spontaneous ' cure ' consequent on the low goitrogenic potency of the cabbage during the months of January to April But this ' cure ' was not complete and the enlargements of the thyroid gland that remained were still considerable and of sufficient magnitude for our purpose

In another place (McCarrison and Madhava, 1932*a*, 1933) it has been shown that co-existing with the thyroid enlargement caused in albino rats by the ' goitre-noxa ' associated with insanitary condition, there is a significant increase in size of the adrenal glands and spleen and a significant diminution in size of the testes and thymus It was desired, therefore, to learn whether the thyroid enlargement caused by another ' goitre-noxa '—that present in cabbage during certain seasons of the year—was associated with similar changes in size of these organs It would appear from the results recorded in this paper that it was not, and that although the two goitre-producing agencies—insanitary condition and cabbage diet—had a similar action on the thyroid gland their action on other organs of the body differed

The experiment.

The experiment was carried out in rabbits It extended over a period of 12 months—from April 1931 to April 1932 The rabbits were divided into two groups the one being fed on the stock diet of raw cabbage, carrots, bran, sprouted Bengal *gram* (legume), green grass and water, and the other on fresh, raw cabbage and water The cabbage given to both groups was obtained from the same source, but that given to the second group was always fresh while that given to the first group was less so It has been noted that if rabbits fed on an exclusive diet of cabbage are to maintain their body-weight the cabbage used must be fresh Each animal was confined in a separate, screened cage under conditions of scrupulous cleanliness During the course of the experiment a few, amongst those fed exclusively on cabbage, died. The results here dealt with relate to the 13 survivors and to 10 control animals fed on stock diet.

Results of the experiment

The results of the experiment are given in Tables I and II wherein the body-weights of the animals in the two groups are set out together with the actual weights and the sizes (the 'r'-values weight per kilogram of body-weight) of various organs of the body In Table III the frequency distributions of the organ-sizes ('r') are shown for the two groups—controls and cabbage-fed

TABLE I

Showing the body-weights and weights of organs in control and in cabbage-fed rabbits

		Number	Body weight, g	Thyroid weight, mg	Adrenal weight, g	Thymus weight, g	Testes weight, g	Spleen weight, g	Liver weight, g	Kidney-weight, g	Heart weight, g
CONTROL	Males	1	1,700	115 2	0 485	2 63	1 62	0 49	61 4	13 15	3 72
		2	1,710	75 8	0 39	0 62	3 40	0 75	68 0	10 6	3 85
		3	1,905	91 2	0 40	1 32	5 75	0 82	86 0	15 5	4 62
		4	2,060	104 6	0 49	1 32	2 84	0 65	79 0	17 2	4 25
		5	1,640	173 2	0 35	0 64	1 10	0 45	65 5	11 1	3 75
		Mean	1,803	112 0	0 43	1 31	3 54	0 63	72 0	13 5	4 04
	Females	6	1,380	101 4	0 35	1 10		0 40	49 0	9 85	3 10
		7	1,850	88 1	0 49	1 92		0 84	93 0	11 2	4 90
		8	1,465	90 4	0 65	0 85		0 55	66 5	10 85	4 35
		9	1,695	50 6	0 44	1 25		0 35	73 0	13 2	4 72
		10	1,470	84 3	0 35	0 22		0 85	58 0	12 2	3 75
		Mean	1,572	83 0	0 46	1 07		0 60	67 9	11 5	4 16
	Mean of all control rabbits		1,688	97 5	0 44	1 19	3 54	0 615	69 9	12 5	4 10

TABLE I—*concld*

		Number	Body-weight, g	Thyroid-size, mg	Adrenal-weight, g	Thymus-weight, g	Testes-weight, g	Spleen-weight, g	Liver-weight, g	Kidney-weight, g	Heart-weight, g
CABBAGE.	Males	11	1,730	719 6	0 65	0 45	1 92	0 52	102 0	14 82	4 42
		12	1,655	354 4	0 55	1 35	2 55	0 60	89 0	13 65	4 25
		13	1,510	563 2	0 40	1 15	5 81	1 52	63 7	14 20	4 30
		14	1,485	249 2	0 31	0 82	3 72	0 62	72 0	10 74	3 50
		15	1,705	400 2	0 43	0 85	3 82	0 52	72 0	11 50	4 25
		16	1,620	361 8	0 75	0 65	3 25	0 67	79 0	10 24	3 27
		17	1,830	550 0	0 53	0 97	3 85	0 45	94 0	15 70	4 15
		18	1,810	584 6	0 35	1 64	2 15	0 85	104 0	12 25	4 14
		Mean	1,668	472 9	0 50	0 985	3 38	0 72	84 5	12 89	4 03
	Females	19	1,710	315 8	0 40	1 38		0 70	57 5	10 8	3 56
		20	1,950	434 2	0 53	1 02		0 82	99 1	12 4	4 50
		21	1,750	424 2	0 34	0 85		1 12	74 0	15 5	4 30
		22	1,775	282 4	0 15	0 65		0 65	64 0	11 5	3 95
		23	1,760	392 8	0 42	1 15		0 68	86 0	11 85	4 55
		Mean	1,789	369 9	0 37	1 01		0 79	76 1	12 4	4 17
	Mean of all cabbage-fed rabbits		1,715	433 3	0 45	0 99	3 38	0 75	81 3	12 7	4 09

TABLE II

Showing the organ-sizes ('r') in control and in cabbage-fed rabbits

		Number	Body weight, g	Thyroid size, mg	Adrenal size, g	Thymus size, g	Testes size, g	Spleen size, g	Liver size, g	Kidney size, g	Heart size, g
CONTROL	Males	1	1,700	68	0 285	1 55	0 95	0 29	36 1	7 7	2 19
		2	1,710	44	0 228	0 36	1 99	0 44	39 8	6 2	2 25
		3	1,905	47	0 210	0 69	3 02	0 43	45 1	8 1	2 43
		4	2,060	51	0 238	0 61	1 38	0 31	38 4	8 4	2 06
		5	1,640	106	0 213	0 39	2 50	0 27	39 9	6 8	2 29
		Mean	1,803	63	0 235	0 72	1 97	0 35	39 9	7 5	2 24
	Females	6	1,380	74	0 253	0 80		0 29	35 5	7 1	2 25
		7	1,850	48	0 264	1 04		0 45	50 3	6 1	2 64
		8	1,465	62	**0·444***	0 58		0 37	45 4	7 4	2 97
		9	1,695	30	0 260	0 74		0 21	43 1	7 8	2 79
		10	1,470	57	0 238	0 15		0 58	39 5	8 3	2 55
		Mean	1,572	54	0 292	0 66		0 38	42 8	7 3	2 64
	Mean of all control rabbits		1,688	58 7	0 263	0 69	1 97	0 36	41 3	7 4	2 44
CABBAGE	Males	11	1 730	415	0 38	0 26	1 11	0 31	59 0	8 56	2 54
		12	1,655	214	0 33	0 82	1 54	0 36	53 7	8 25	2 26
		13	1,510	372	0 26	0 76	3 87	**1 01***	42 2	9 40	2 84
		14	1,485	167	0 22	0 57	2 50	0 42	48 5	7 23	2 36
		15	1,705	236	0 25	0 50	2 24	0 30	39 2	6 75	2 49
		16	1,620	222	0 46	0 40	2 01	0 41	48 8	6 32	2 02
		17	1 830	300	0 30	0 53	2 10	0 25	51 4	8 58	2 27
		18	1 810	321	0 19	0 91	1 19	0 47	57 5	5 18	2 28
		Mean	1,668	281	0 30	0 59	2 07	0 44	50 0	7 53	2 38
	Females	19	1,710	184	0 23	0 81		0 41	33 6	6 32	2 08
		20	1,950	222	0 27	0 52		0 42	50 8	6 36	2 82
		21	1,750	242	0 19	0 49		0 64	35 4	8 86	2 46
		22	1,775	160	**0 08***	0 37		0 37	36 1	6 48	2 23
		23	1,760	223	0 24	0 65		0 39	48 9	6 73	2 59
		Mean	1,789	206	0 20	0 57		0 44	41 0	6 95	2 44
	Mean of all cabbage fed rabbits		1,715	252 2	0 263	0 58	2 07	0 44	46 5	7 31	2 40

* Possible spurious result

TABLE III

Showing the frequency distribution of organ-sizes ('r') in control and in cabbage-fed rabbits

Organ	Range of organ size 'r'	Control	Cabbage-fed
Liver	30—39 g	6	4
	40—49 g	3	4
	50—59 g	1	5
Testes	0 0—0 9 g	1	
	1 0—1 9 g	2	3
	2 0—2 9 g	1	4
	3 0—3 9 g	1	1
Kidney	5 0—5 9 g		1
	6 0—6 9 g	3	6
	7 0—7 9 g	4	1
	8 0—8 9 g	3	4
	9 0—9 9 g		1
Heart	2 00—2 19 g	2	2
	2 20—2 39 g	3	5
	2 40—2 59 g	2	4
	2 60—2 79 g	2	
	2 80—2 99 g	1	2
Adrenal	0 00—0 09 g		1
	0 10—0 19 g		2
	0 20—0 29 g	9	6
	0 30—0 39 g		3
	0 40—0 49 g	1	1
Thymus	0 10—0 39 g	3	2
	0 40—0 69 g	3	7
	0 70—0 99 g	2	4
	1 00—1 29 g	1	
	1 30—1 59 g	1	
Thyroid	30—69 mg	8	
	70—109 mg	2	
	110—149 mg		
	150—189 mg		3
	190—229 mg		4
	230—269 mg		2
	270—309 mg		1
	310—349 mg		1
	350—389 mg		1
	390—429 mg		1
Spleen	0 20—0 29 g	4	1
	0 30—0 39 g	2	5
	0 40—0 49 g	3	5
	0 50—0 59 g	1	
	0 60—0 69 g		1
	1 00—1 09 g		1

The mean values of ' r ', together with the significance, or want of significance, of difference in the two groups are as under —

Organ	Controls	Cabbage fed	Difference	Significance
Thyroid	58 7 ± 6 6	252 2 ± 21 6	193 5 ± 22 6	t = 8 6, significant
Testes	1 97	2 07	0 10	Not significant
Spleen	0 36	0 44	0 08	„ „
Liver	41 3	46 5	5 2	„ „
Adrenals	0 263	0 263	*Nil*	
Kidneys	7 4	7 3	0 1	„ „
Heart	2 44	2 40	0 04	„ „
Thymus	0 69	0 58	0 11	„ „

These results are to be anticipated from the frequency distributions of organ-sizes (' r ') The distributions for all organs, except the thyroid gland, largely

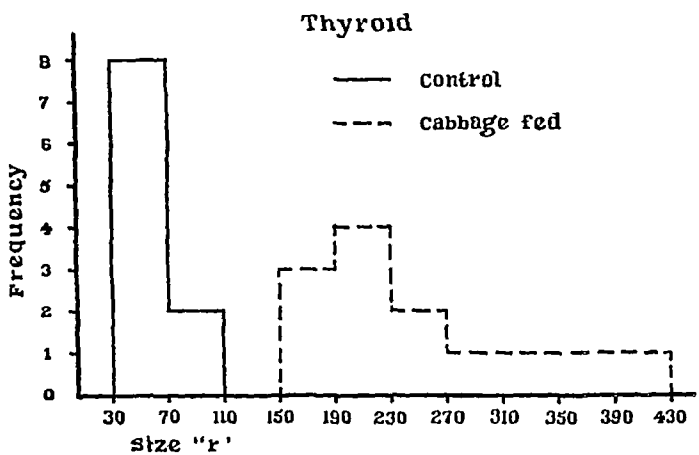

Fig 1 Showing the frequency distribution of thyroid sizes in normal, well fed rabbits (control) and in rabbits fed exclusively on cabbage

overlap in the two groups The thyroid distribution shows a high degree of positive skewness, indicative of the occurrence of goitres, in the cabbage-fed animals (Fig 1)

The goitrogenic action of the cabbage diet is thus conclusively demonstrated It will be noted that the testes, spleen and liver are slightly larger in the cabbage-fed group than in controls, but the excess is not significant The kidneys, heart and adrenals have almost identical values in the two groups, and such slight differences as exist are of no significance The thymus tends to be smaller than in controls, but here, again, the diminution in its size is not significant

Possible 'spurious values', included in these results are those shown in bold type in Table II There are two amongst the adrenal-sizes—one large and one small value—and one unusually high value amongst the spleen-sizes But even when these are excluded the same lack of significance in the difference between the two groups is maintained Thus, excluding the two unusual adrenal-sizes, the mean 'r'-values are as follows —

Controls	0 2432 ± 0 0082
Cabbage-fed	0 2767 ± 0 0231

and the difference between them (0 0335 ± 0 0245) is not significant Nevertheless, by the exclusion of these two unusual values the adrenal glands fall into the category of organs exhibiting a tendency, though slight and not statistically significant, to enlargement in the cabbage-fed animals

It is apparent from these results that the goitrogenic agent in cabbage did not bring about, within a period of one year of exposure to it, any statistically significant change in the weight or size of organs other than the thyroid gland This result is in marked contrast to that observed in rats exposed, for a period of only 120 days, to the goitrogenic influence of insanitary condition (McCarrison and Madhava, 1932*a*, 1933), in these animals significant changes in size occurred in the adrenal glands, the spleen, the testes and the thymus, as well as in the thyroid gland

It is obvious, also, that the goitre produced by the goitrogenic agent in cabbage did not cause any increase in size of the heart 'goitre-heart' was not a concomitant of 'cabbage goitre', nor, as shown in another place (McCarrison and Madhava, 1932*a*), was it a concomitant of the goitres associated with insanitary condition

The results of the experiment are distinguished by sex in Tables I and II, it will be noted from them that sex-differences were trivial

H ion activity of the blood and organs.

Determinations of pH were made by means of the glass-electrode The technicalities relating to the use of this electrode are dealt with in another paper in this issue (Sankaran, 1933) The animals were killed by air-embolism, and their organs were removed and examined as soon after death as possible, organs awaiting

their turn for examination being kept meanwhile in cold storage All examinations were completed within 3 hours of the death of the animal Spurious results, liable to arise in consequence of post-mortem changes, were thus avoided The blood used was withdrawn direct from the heart immediately before death

TABLE IV

Showing the pH values of blood and organs in control and in cabbage-fed rabbits

		Number	Blood	Thyroid	Adrenal	Thymus	Testes	Spleen	Liver	Kidney	Heart	Brain	Pancreas
CONTROL	Males	1	7 68	6 95	6 80	6 85		6 78	6 76	6 42		6 59	**7·26***
		2	7 61	6 96	6 72	6 82	6 62	6 48	6 44	6 55	6 05	6 57	6 79
		3	7 61	6 91	6 68		6 55	6 37	6 44	6 55	6 12	6 52	6 65
		4	7 66	6 86	6 65	6 76	6 59	6 48	6 11	6 48	6 05	6 55	
		5	7 62	6 86	6 62	6 86	6 52	6 52	6 48	6 28	6 08	6 48	
		Mean	7 64	6 91	6 69	6 82	6 57	6 53	6 51	6 46	6 08	6 54	6 72
	Females	6	7 68	**7 12***	6 92	6 88		6 81	6 62	6 62	5 95	6 57	6 85
		7	7 62	6 91	6 65	6 65		6 39	6 30	6 48	5 99	6 48	6 65
		8	7 57	6 89	6 62	6 89		6 44	6 48	6 52	6 12	6 59	6 62
		9	7 52	6 89	6 55	6 62		6 35	6 20	6 28	5 47	6 48	
		10	7 61	6 91	6 55	6 82		6 41	6 33	6 33	5 84	6 54	
		Mean	7 60	6 90	6 66	6 77		6 48	6 39	6 45	5 87	6 53	6 71
	Male and female together		7 62	6 90	6 68	6 79	6 57	6 50	6 45	6 45	5 96	6 54	6 71

* Possible spurious result

TABLE IV—*concld*

			Number	Blood	Thyroid	Adrenal	Thymus	Testes	Spleen	Liver	Kidney	Heart	Brain	Pancreas
CABBAGE-FED	Males		11	7 61	7 00	6 75	6 93	6 75	6 71	6 36	6 48	5 90	6 59	.
			12	7 64	7 00	6 75	6 93	6 65	6 61	6 44	6 54	5 84	6 61	6 79
			13	7 64	7 10	6 87		6 75	6 70	6 52	6 61	5 90	6 58	
			14	7 65	7 05	6 65	7 10	6 79	6 67	6 30	6 62	5 96		7 18
			15	7 60	7 10	6 74	7 09	6 65	6 51	6 43	6 51	6 15	6 78	7 02
			16	7 65	7 05	6 70	7 07	6 68	6 54	6 37	6 32	6 08	6 26	6 65
			17	7 64	7 12	6 65	6 61	6 75	6 51	6 30	6 40	5 80	6 65	6 78
			18	7 53	6 91	6 73	7 00	6 65	6 48	6 31	6 41	5 73	6 58	7 08
			Mean	7 62	7 06	6 73	6 96	6 71	6 59	6 38	6 49	5 92	6 57	6 92
	Females		19	7 64	7 00	6 65	7 93		6 58	6 36	6 43	5 91	6 65	7 00
			20	7 67	7 04	6 69	7 05		6 58	6 37	6 47	5 86		6 54
			21	7 60	7 03	6 69	6 96		6 35	6 19	6 44	5 86	6 51	6 72
			22	7 64	7 07	6 82	6 47		6 47	6 12	6 47	6 12	6 44	7 31
			23	7 60	7 00	6 79	7 10		6 44	6 37	6 30	5 76	6 55	6 58
			Mean	7 63	7 03	6 73	7 10		6 48	6 28	6 42	5 91	6 54	6 83
	Male and female together			7 62	7 05	6 73	7 02	6 71	6 55	6 34	6 46	5 92	6 56	6 88

TABLE V

Showing the frequency distribution of pH values in control and cabbage-fed rabbits

Range of pH value	Blood	
	Control	Cabbage
7 50—7 59	2	1
7 60—7 69	9	12
	Heart	
5 40—5 49	1	
5 70—5 79	1	2
5 80—5 89	1	4
5 90—5 99	2	4
6 00—6 09	3	1
6 10—6 19	2	2

Range of pH value	Thymus		Pancreas		Testes		Adrenals		Thyroid	
	Control	Cabbage	Control	Cabbage	Control	Cabbage	Control	Cabbage	Control	Cabbage
6 40—6 49		1								
6 50—6 59				2	3		2			
6 60—6 69	2	1	3	1	1	4	5	5		
6 70—6 79	1		1	3		4	1	6		
6 80—6 89	6		1				1	2	4	
6 90—6 99		3					1		5	1
7 00—7 09		4		3						8
7 10—7 19		2		1					1*	4
7 20—7 29			1*							
7 30—7 39				1						
7 90—7 99		1								

* Possible spurious result

TABLE V—*concld*

Range of pH value	LIVER		KIDNEY		SPLEEN		BRAIN	
	Control	Cabbage	Control	Cabbage	Control	Cabbage	Control	Cabbage
6 10—6 19		2						
6 20—6 29	1		2					1
6 30—6 39	2	8	1	2	3	1		
6 40—6 49	5	2	3	7	4	3	3	1
6 50—6 59	.	1	3	2	1	5	7	5
6 60—6 69	1		1	2		2		3
6 70—6 79	1				1	2		1
6 80—6 89					1			

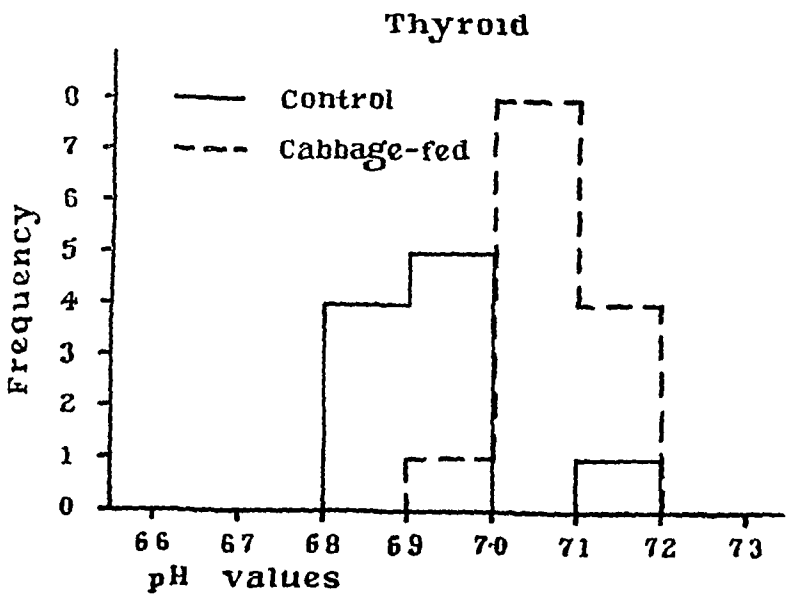

Fig 2. Showing frequency distribution of pH values of the thyroid gland in normal, well-fed rabbits (control) and in rabbits fed exclusively on cabbage.

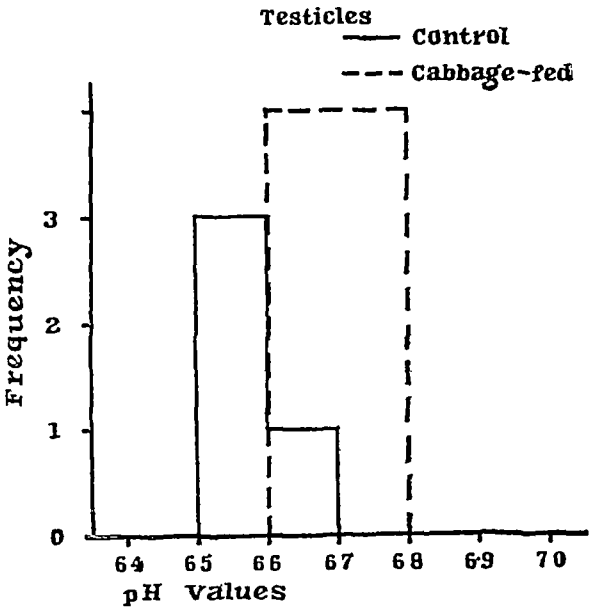

Fig 3 Showing frequency distribution of pH values of the testes in normal, well fed rabbits (control) and in rabbits fed exclusively on cabbage

The results of the pH determinations are given in Table IV and in frequency groups in Table V

The mean values are as under —

		Controls	Cabbage fed	Difference	Significance
1	Blood	7 62	7 62	*Nil*	
2	Thyroid	6 90 ± 0 0116	7 05 ± 0 0125	0 15 ± 0 0171	t = 8 8, significant
3	Thymus	6 79 ± 0 0328	7 02 ± 0 1059	0 23 ± 0 1059	t = 2 2, not significant
4	Pancreas	6 71 ± 0 0454	6 88 ± 0 0769	0 17 ± 0 0893	t = 1 9 ,, ,,
5	Adrenal	6 68	6 73	0 05	,, ,,
6	Testes	6 57 ± 0 0220	6 71 ± 0 0197	0 14 ± 0 0295	t = 4 7, significant
7	Brain	6 54	6 56	0 02	Not significant
8	Spleen	6 50	6 55	0 05	,, ,,
9	Liver	6 45 ± 0 0503	6 34 ± 0 0288	0 11 ± 0 0580	t = 1 9, not significant
10	Kidney	6 45	6 46	0 01	,, ,,
11	Heart	5 96	5 92	0 04	,, ,,

It will be observed from these results that while the mean pH of the blood is the same in both groups that of the thyroid, thymus, pancreas, adrenal, testes and spleen tends to be higher in the cabbage-fed group But the differences between the two groups are not significant, except in the case of the thyroid gland and testes when they are definitely so Possible 'spurious values' are indicated in bold type in Table IV, their omission does not, however, effect the significance of the results tabulated above

The results are distinguished by sex in Table IV, such differences as exist in the two sexes are trivial

The pH values are, as a rule, most constant, the co-efficients of variability being amongst the lowest on record in any biometric investigation in this Laboratory These co-efficients are as follows —

Organ	Controls	Cabbage fed
Blood	0 64 per cent	0 46 per cent
Thyroid	0 50 ,,	0 80 ,,
Thymus	1 45 ,,	4 97 ,,
Pancreas	1 52 ,,	3 71 ,,
Adrenals	1 71 ,,	1 01 ,
Testes	0 67 ,,	0 83 ,,
Brain	0 68 ,,	2 02 ,,
Spleen	2 50 ,,	1 62 ,,
Liver	2 47 ,,	1 63 ,,
Kidney	1 85 ,,	1 48 ,,
Heart	3 44 ,,	2 24 ,,
Average	1 58 per cent	1 88 per cent

From these results it is seen that the only organs, amongst those examined, whose H ion activity is altered to any significant degree by the cabbage diet, are the thyroid gland and the testes, in both these organs the increased alkalinity is associated with an increase in size of the organ—significant in the former but not in the latter

Relation between size ('r') and pH of organs

A partial answer to the question of a possible relation between 'r' and pH is afforded by the above results (1) There is no significant difference in the pH values of the thymus, liver, heart, kidney, spleen and adrenal glands in the two groups, nor is there any significant difference in their sizes (2) There is a significant excess in the pH values of the thyroid and testes in the cabbage-fed group, but the increase in size ('r') associated with the greater alkalinity is significant only in the thyroid The correlation co-efficients between 'r' and pH for the several organs provide the complete answer These co-efficients are as follows —

Organ	Correlation co efficients	Correlation
Heart	− 0 2562 ± 0 1344	*Nil*
Liver	− 0 1875 ± 0 1357	*Nil*
Spleen	− 0 0024 ± 0 1406	*Nil*
Adrenals	− 0 1166 ± 0 1387	*Nil*
Kidneys	+ 0 1690 ± 0 1366	*Nil*
Thymus	+ 0 3225 ± 0 1386	*Nil*

Summary.

1 Two groups of rabbits, of approximately the same initial body-weight, were employed in this investigation, the one (controls) was fed on the stock diet in use for rabbits in this Laboratory, the other on an exclusive diet of fresh, raw cabbage and water The experiment was continued for a period of 12 months

2 On its conclusion the mean weight of the thyroid gland, both sexes taken together, was found to be 97 5 mg in controls and 433 3 mg in those fed exclusively on cabbage, the mean size of the organ was 58 7 mg per kilogram of body-weight in controls and 252 2 mg in those fed exclusively on cabbage The differences between the two groups are significant and the goitre-producing action of cabbage is again demonstrated

3 The thyroid enlargement, brought about by the cabbage diet, was not associated with any statistically significant change in size of other organs of the body (testes, spleen, adrenals or thymus), differing in this respect from the thyroid enlargement brought about in albino rats by insanitary condition The pituitary body and pancreas were not examined from this point of view

4 The H ion activity of the blood was not altered as a result of exclusive feeding on cabbage, nor was that of the adrenal glands, the spleen, the brain, the

kidney and the heart The liver tended to be more acid but not to a degree which was statistically significant The thyroid, the testes, the pancreas and the thymus were more alkaline but only to a significant degree in the case of the thyroid gland and testes

5 The thyroid gland was the only organ, amongst those examined, in which a correlation existed between size and H ion concentration

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Ind Jour Med Res, XX, 3, *January*, 1933

HYDROGEN ION CONCENTRATION IN THE ORGANS OF PIGEONS FED ON POLYNEURITIS-PRODUCING DIETS

BY

R McCARRISON, G SANKARAN AND K B MADHAVA *
(*From the Nutritional Research Laboratories, I R F A, Coonoor*)

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Material.

THE material for this investigation was provided by five groups (I to V) of 21 pigeons each Groups I and II were fed on well-constituted 'Control diets', Groups III, IV and V on diets deficient in vitamin B Details of these diets are as follows —

A CONTROL DIETS

I A diet of mixed grain *cholam* (*Andropagan sorghum*), 6 parts, *mung dhal* (*Phaseolus mungo*), 1 part, *ragi* (*Eleusine coracana*), 1 part, *cambu* (*Pennisetum typhoideum*), 2 parts, water *ad libitum*

II A diet of washed, raw, polished rice, each bird receiving in addition 5 g of *dhal arhar* (*Cajanus indicus*), 5 drops of cod-liver oil and 10 drops of orange juice, daily These additions were administered artificially every morning Water *ad libitum*

B DEFICIENT DIETS

III A diet of autoclaved mixed grains This diet was of the same composition as I, but was autoclaved in an alkaline medium for an hour-and-a-half at 130°C Water *ad libitum*

IV A diet of the same composition as II but in which the *dhal arhar* was autoclaved The autoclaved *dhal*, cod-liver oil and orange juice were administered artificially Water *ad libitum*

V An exclusive diet of the same washed, raw, polished rice as that used in diets II and IV Water *ad libitum*

* The first author is responsible for the experimental data, the second for the data of pH determinations and the third for the statistical analysis of these data —R McC

The mean body-weight of the birds (Table I) and the distributions of body-weights around the mean were approximately the same in each group The pigeons were confined in roomy, screened cages—three to a cage—under conditions of scrupulous cleanliness

The experiment was carried out during the hot, dry months of the year (March to June, 1932) when the rainfall was practically nil The incidence of *beri-beri columbarium*, as opposed to the *polyneuritis columbarum* (McCarrison, 1928), was, accordingly, low Thus, the diet of autoclaved mixed grains was not associated in this experiment with the occurrence of typical beri-beri, whereas a diet of the same composition gave rise to an incidence of 36 6 per cent during the North-East Monsoon (McCarrison, 1928) Similarly, only one case of typical beri-beri (or 5 per cent) occurred in Group IV, whereas a diet of the same composition caused typical beri-beri in 17 4 per cent of pigeons in Rangoon (Taylor and Thant, 1929) Climatic and regional influences play an important part in the causation of true beri-beri both in birds, under experimental conditions, and in human beings

The experiment was continued until all the deficiently-fed birds had died or been killed at the onset of polyneuritis A complete post-mortem examination of each bird was made as soon after death as possible, the organs being rapidly removed, weighed, and transferred to the cold chamber in order to avoid the alterations in pH which result from post-mortem changes When all deficiently-fed birds had been so dealt with the controls were then examined in the same way The blood needed for pH determinations was taken from the wing veins of the living birds All estimations of hydrogen ion concentration were made with the Glass-Electrode, details regarding the use of which, and the precautions needed in its use, are given in the preceding paper

Organ-weights and sizes.

The mean weight of the organs and their proportionate size in the body ('r'= weight per 100 g of body-weight) are set out in Table I, together with the average number of days of survival of the birds in each group In this and in succeeding Tables (II and III) the birds that were killed (designated 'K') are separated from those that died (designated 'D') Table I brings into prominence the effect of the deficient diets in causing atrophy of the spleen and testes and hypertrophy of the adrenal glands (McCarrison, 1919) The abnormally large weight and size ('r') of the thyroid gland in the group (V) fed exclusively on washed, polished rice is an unusual finding which will be dealt with in another place

Results of pH determinations.

The results of pH determinations are given as mean values in Table II, while in Table III the statistical constants for each organ, tissue or fluid examined are set out together with the number of observations made in each case

TABLE I

Showing the body-weights, organ-weights and organ-sizes ('r'=weight per 100 g of body-weight)

Group number	I	II	III			IV			V			Controls I+II	Deficiently-fed III+IV+V
Killed or died	Killed	Killed	K+D	Killed	Died	K+D	Killed	Died	K+D	Killed	Died	Killed	K+D
Number of birds	20*	21	21	7	14	20*	6	14	21	6	15	41	62
Days of experiment	73	87	19	59	43	38	36	40	40	40	40	80	42
Initial body weight, g	274	273	275	278	273	271	278	268	275	279	273	273	274
Final body weight, g	270	297	173	176	172	182	200	175	173	187	168	283	176
Organ weights													
Heart, g	3 08	3 30	2 08	2 01	2 11	2 21	2 45	2 11	2 28	2 27	2 28	3 19	2 19
Liver, g	5 66	7 03	4 68	3 69	5 17	5 21	4 64	5 45	5 73	5 58	5 78	6 34	5 20
Spleen, mg	230	260	79	52	94	77	101	66	77	103	66	245	78
Pancreas, g	0 78	0 83	0 49	0 45	0 51	0 53	0 57	0 51	0 57	0 60	0 56	0 80	0 53
Testes, g	1 49	1 05	0 10	0 86	0 10	0 11	0 86	0 12	0 19	0 11	0 22	1 27	0 13
Thyroid, mg	20 8	20 9	13 8	13 6	13 9	14 3	17 3	13 0	30 6	29 7	31 0	20 9	19 6
Adrenals, mg	39 0	36 5	48 0	41 3	51 9	62 0	54 0	66 0	56 0	57 0	55 0	37 7	55 3
Organ sizes ('r')													
Heart, g	1 11	1 12	1 20	1 11	1 25	1 22	1 23	1 21	1 32	1 20	1 37	1 12	1 25
Liver, g	2 10	2 37	2 70	2 01	3 04	2 90	2 33	3 14	3 34	2 97	3 49	2 24	2 98
Spleen, mg	89 2	86 0	37 7	29 0	55 0	47 4	50 1	44 0	39 6	58 8	40 0	87 6	41 6
Pancreas, g	0 28	0 27	0 28	0 24	0 31	0 29	0 30	0 30	0 33	0 32	0 33	0 27	0 30
Testes, g	0 53	0 35	0 05	0 05	0 06	0 06	0 04	0 07	0 06	0 05	0 07	0 44	0 06
Thyroid, mg	7 73	7 08	8 10	7 73	8 49	7 92	8 68	7 60	17 57	15 82	18 27	7 40	11 20
Adrenals, mg	14 5	12 3	27 8	22 4	30 5	35 3	27 2	39 1	32 5	30 5	33 3	13 4	31 87

* One bird excluded

TABLE II

Showing the mean pH values

GROUP.	I	II	III		IV		V		CONTROLS I + II	DEFICIENTLY FED III + IV + V
Killed or died	Killed	Killed	Killed	Died	Killed	Died	Killed	Died	Killed	Killed
Blood	7 63	7 66	7 65		7 69		7 56	7 69	7 64	7 63
Pericardial fluid				7 69		7 11	7 34	7 21		7 34
Thyroid gland	6 91	6 99	6 82	7 35	6 88	7 09	7 33	6 93	6 95	7 01
Optic lobes	6 87	6 86	6 17	6 54	6 06	6 22	6 44	6 65	6 86	6 22
Sciatic nerve	6 87	6 90	6 74	7 11	6 82	6 98	7 01	7 04	6 88	6 86
Pons	6 84	6 84	6 12	6 60	6 18	6 27	6 39	6 84	6 84	6 23
Cerebellum	6 78	6 80	6 17	6 52	6 25	6 24	6 52	6 64	6 79	6 32
Cerebrum	6 69	6 70	5 85	6 20	5 94	6 03	6 22	6 32	6 69	6 00
Pancreas	6 61	6 59	6 52	6 51	6 44		6 57	6 55	6 60	6 51
Spleen	6 56	6 50	6 44	6 68	6 51	6 58	6 59	6 62	6 53	6 51
Testes	6 53	6 55		6 98	6 12	6 46		6 89	6 54	6 12
Adrenal glands	6 47	6 46	6 20	6 65	6 31	6 68	6 32	6 63	6 46	6 28
Kidney	6 41	6 37	6 44		6 41	6 64	6 15	6 38	6 39	6 33
Heart, auricle	6 39	6 29	6 42	6 91	6 21	6 87	6 65	7 01	6 34	6 43
Heart, ventricle	6 23	6 11	6 26	6 62	5 90	6 32	6 22	6 39	6 17	6 13
Liver	6 08	6 04		6 35	6 36	6 50	6 27	6 30	6 06	6 31
Limb muscle	5 83	5 88	6 62	6 72	6 23	6 82	6 77	6 80	5 85	6 54
Chest muscle	5 78	5 69	6 52	6 68	6 16	7 00	6 76	6 66	5 69	6 48

TABLE III

Showing mean pH with standard error, standard deviation σ and co-efficient of variation

Organ, tissue or fluid	Group or sub group	Number of observations	Mean pH with standard error	σ	Co efficient of variation
Blood	I	19	7 63 ± 0 01	0 05	0 7
	II	21	7 66 ± 0 02	0 08	1 0
	III K	6	7 65 ± 0 03	0 06	0 8
	IV K	4	7 69 ± 0 05	0 09	1 2
	V K	4	7 56 ± 0 07	0 13	1 7
	V D	1	7 69		
Pericardial fluid	III D	1	7 69		
	IV D	4	7 11 ± 0 27	0 53	7 5
	V K	1	7 34		
	V D	5	7 21 ± 0 20	0 44	6 1
Thyroid	I	15	6 91 ± 0 02	0 08	1 2
	II	21	6 99 ± 0 01	0 07	1 0
	III K	1	6 82		
	III D	2	7 35 ± 0 24	0 35	4 7
	IV K	4	6 88 ± 0 22	0 45	6 5
	IV D	5	7 09 ± 0 11	0 25	3 6
	V K	2	7 33 ± 0 16	0 23	3 1
	V D	6	6 93 ± 0 24	0 59	8 5
Optic lobes	I	19	6 87 ± 0 02	0 09	1 3
	II	21	6 86 ± 0 01	0 06	0 9
	III K	7	6 17 ± 0 10	0 26	4 2
	III D	12	6 54 ± 0 11	0 37	5 7
	IV K	6	6 06 ± 0 13	0 31	5 1
	IV D	13	6 22 ± 0 19	0 68	10 9
	V K	6	6 44 ± 0 16	0 37	5 8
	V D	15	6 65 ± 0 12	0 46	6 9

TABLE III—*contd*

Organ, tissue or fluid	Group or sub group	Number of observations	Mean pH with standard error	σ	Co efficient of variation
Sciatic nerve	I	19	6 87 ± 0 01	0 05	0 7
	II	21	6 90 ± 0 02	0 08	1 1
	III K	6	6 74 ± 0 11	0 26	3 9
	III D	12	7 11 ± 0 07	0 24	3 4
	IV K	5	6 82 ± 0 10	0 21	3 1
	IV D	12	6 98 ± 0 12	0 41	5 9
	V K	16	7 01 ± 0 11	0 27	3 8
	V D	14	7 04 ± 0 07	0 26	3 7
Pons	I	19	6 84 ± 0 01	0 05	0 7
	II	21	6 84 ± 0 02	0 08	1 2
	III K	7	6 12 ± 0 11	0 29	4 8
	III D	12	6 60 ± 0 10	0 35	5 3
	IV K	6	6 18 ± 0 10	0 25	4 0
	IV D	13	6 27 ± 0 18	0 65	10 3
	V K	6	6 39 ± 0 12	0 29	4 6
	V D	14	6 84 ± 0 12	0 47	6 8
Cerebellum	I	19	6 78 ± 0 02	0·07	1 0
	II	21	6 80 ± 0 02	0 07	1 0
	III K	7	6 17 ± 0 09	0 25	4 1
	III D	12	6 52 ± 0 10	0 33	5 1
	IV K	6	6 25 ± 0 16	0 40	6 5
	IV D	13	6 24 ± 0 18	0 65	10 5
	V K	6	6 52 ± 0 08	0 19	2 9
	V D	15	6 64 ± 0 10	0 39	5 9

TABLE III—*contd*

Organ, tissue or fluid	Group or sub group	Number of observations	Mean pH with standard error	σ	Co efficient of variation
Cerebrum	I	19	6 69 ± 0 02	0 09	1 4
	II	21	6 70 ± 0 02	0 08	1 2
	III K	7	5 85 ± 0 10	0 26	4 4
	III D	12	6 20 ± 0 10	0 34	5 5
	IV K	6	5 94 ± 0 12	0 29	4 9
	IV D	12	6 03 ± 0 16	0 57	9 4
	V K	6	6 22 ± 0 10	0 25	4 1
	V D	15	6 32 ± 0 12	0 46	7 2
Pancreas	I	16	6 61 ± 0 02	0 07	1 0
	II	20	6 59 ± 0 02	0 07	1 0
	III K	1	6 52		
	III D	2	6 51 ± 0 18	0 25	3 8
	IV K	5	6 44 ± 0 06	0 12	1 9
	V K	2	6 57 ± 0 13	0 18	2 8
	V D	7	6 55 ± 0 11	0 28	4 2
Spleen	I	19	6 56 ± 0 02	0 07	1 1
	II	21	6 50 ± 0 02	0 09	1 4
	III K	6	6 44 ± 0 11	0 26	4 1
	III D	5	6 68 ± 0 20	0 45	6 7
	IV K	6	6 51 ± 0 05	0 12	1 8
	IV D	7	6 58 ± 0 18	0 48	7 3
	V K	4	6 59 ± 0 06	0 12	1 9
	V D	10	6 62 ± 0 15	0 46	7 0

TABLE III—*contd*

Organ, tissue or fluid	Group or sub group	Number of observations	Mean pH with standard error	σ	Co efficient of variation
Testes	I	10	6 53 ± 0 03	0 08	1 2
	II	15	6 55 ± 0 02	0 07	1 1
	III D	1	6 98		
	IV K	1	6 12		
	IV D	2	6 46 ± 0 36	0 51	7 9
	V D	3	6 89 ± 0 08	0 14	2 0
Adrenals	I	19	6 47 ± 0 02	0 09	1 4
	II	21	6 46 ± 0 02	0 10	1 5
	III K	6	6 20 ± 0 08	0 19	3 1
	III D	8	6 65 ± 0 12	0 33	5 0
	IV K	6	6 31 ± 0 06	0 16	2 5
	IV D	10	6 68 ± 0 13	0 40	6 0
	V K	5	6 32 ± 0 13	0 28	4 5
	V D	10	6 63 ± 0 13	0 42	6 3
Kidney	I	16	6 41 ± 0 03	0 10	1 6
	II	21	6 37 + 0 03	0 15	2 4
	III K	1	6 44		
	IV K	5	6 41 ± 0 09	0 19	3 0
	IV D	2	6 64 ± 0 01	0 02	0 3
	V K	2	6 15 ± 0 01	0 01	0 2
	V D	6	6 38 ± 0 18	0 43	6 7

TABLE III—*contd*

Organ, tissue or fluid	Group or sub group	Number of observations	Mean pH with standard error	σ	Co efficient of variation
Heart, auricle	I	19	6 39 ± 0 04	0 16	2 5
	II	21	6 29 ± 0 03	0 12	1 9
	III K	7	6 12 ± 0 12	0 32	5 0
	III D	12	6 91 ± 0 09	0 32	4 6
	IV K	4	6 21 ± 0 04	0 09	1 1
	IV D	12	6 87 ± 0 11	0 38	5 6
	V K	6	6 65 ± 0 20	0 50	7 5
	V D	15	7 01 ± 0 11	0 43	6 2
Heart, ventricle	I	19	6 23 ± 0 04	0 16	2 6
	II	21	6 11 ± 0 03	0 14	2 3
	III K	7	6 26 ± 0 14	0 37	5 9
	III D	11	6 62 ± 0 13	0 43	6 5
	IV K	4	5 90 ± 0 10	0 20	3 5
	IV D	12	6 32 ± 0 12	0 43	6 8
	V K	4	6 22 ± 0 33	0 66	10 6
	V D	13	6 39 ± 0 14	0 51	7 9
Liver	I	16	6 08 ± 0 02	0 10	1 6
	II	21	6 04 ± 0 03	0 14	2 3
	III D	1	6 35		
	IV K	5	6 36 ± 0 12	0 26	4 1
	IV D	2	6 50 ± 0 02	0 03	0 4
	V K	3	6 27 ± 0 10	0 18	2 8
	V D	6	6 30 + 0 20	0·49	7 7

TABLE III—*concld*

Organ, tissue or fluid	Group or sub group	Number of observations	Mean pH with standard error	σ	Co efficient of variation
Muscle (limb)	I	8	5 83 ± 0 07	0 19	3 3
	II	4	5 88 ± 0 14	0 27	4 6
	III K	1	6 62		
	III D	1	6 72		
	IV K	2	6 23 ± 0 21	0 30	4 8
	IV D	1	6 82		
	V K	2	6 77 ± 0 33	0 47	6 9
	V D	5	6 80 ± 0 30	0 68	10 0
Muscle (chest)	I	8	5 78 ± 0 14	0 40	6 9
	II	4	5 60 ± 0 07	0 13	2 3
	III K	2	6 52 ± 0 22	0 31	4 8
	III D	1	6 68		
	IV K	3	6 16 ± 0 20	0 34	5 6
	IV D	1	7 00		
	V K	2	6 75 ± 0 45	0 64	9 4
	V D	5	6 66 ± 0 58	1 30	19 3

pH of normal organs and tissues.

It will be noted from Tables II and III that, with the exception of the heart, the pH of the organs and tissues of the body is practically the same in the two control groups (I and II) These may, therefore, be combined for the purpose of arriving at an estimate of the normal pH The mean values given by this combination are shown in the penultimate column of Table II

The Heart —The pH of the auricle is, as a rule, higher than that of the ventricle Diet II tended to produce greater acidity both in the auricles and in the ventricles

than Diet I The frequency distributions of pH values indicate the extent of these tendencies —

Range of pH	Auricle		Ventricle	
	Diet I	Diet II	Diet I	Diet II
5 8 to 5 89			1	0
5 9 „ 5 99			0	5
6 0 „ 6 09	1		4	4
6 1 „ 6 19	1	4	1	6
6 2 „ 6 29	1	8	6	2
6 3 „ 6 39	9	5	4	4
6 4 „ 6 49	5	4	3	
6 5 „ 6 59	1			
6 6 „ 6 69	0			
6 7 „ 6 79	1			

The actual limits of pH were as follows —

	Auricle	Ventricle
Group I	6 04 to 6 76	5 86 to 6 48
„ II	6 12 „ 6 48	5 90 „ 6 37

It will be noted, from Tables II and III, that in both groups the difference between the mean pH values of the auricle and ventricle is approximately the same 0 16 in Group I and 0 18 in Group II, with a mean value of 0 17 when both groups are taken together But the range of variation in this difference is considerable 0 0 to 0 35 , the maximum being reached, or nearly approached, in only 4 of the 40 birds examined and the minimum in only two It seems probable that cardiac electrotonus is at its optimum within a relatively narrow range of pH and of difference in pH between auricle and ventricle (Lillie, 1924 , Henri Fredericq, 1928)

The sensitivity of the heart to differences in composition of the diet is indicated by the significantly greater acidity of both auricle and ventricle in birds fed on Diet II than in those fed on Diet I

The Blood —The pH of the blood has a mean value of 7 645 in healthy, well-fed pigeons Its range in the two control groups was as follows —

Group I 7 55 to 7 73
„ II 7 55 „ 7 79

It is the highest and at the same time the least variable of all pH values given by the organs and tissues examined (Tables II and III)

The Thyroid Gland is the most alkaline of all organs of the body Its pH ranged between 6 74 and 7 00 in Group I, and between 6 87 to 7 12 in Group II The diet on which the latter group was fed tends, therefore, to the production of slightly—though not significantly—greater alkalinity in the thyroid gland It will be noted also that the thyroid gland was slightly smaller in the latter group (' r '-values in Table I) The co-efficients of variability of pH values of the thyroid are, in both control groups, very low (Table III)

The Liver is the most acid of the organs examined (the gastro-intestinal tract was excluded from this investigation) The range of pH lay between 5 95 and 6 33 in Group I and between 5 77 and 6 33 in Group II

Between these two extremes—the thyroid gland on the one hand and the liver on the other—come the *Pancreas*, *Spleen* and *Testes* which are more acid than the thyroid and less so than the liver though not differing significantly amongst themselves The range of pH values given by these organs in the two control groups was as follows —

	Group I	Group II
Pancreas	6 44 to 6 68	6 48 to 6 68
Spleen	6 48 „ 6 68	6 30 „ 6 65
Testes	6 41 „ 6 64	6 44 „ 6 65

The co-efficients of variability were, in all, of the same low order

Next in order of acidity come the *Adrenal Glands* and *Kidneys* whose pH values are of approximately the same order of magnitude The range of these values is also approximately the same —

	Group I	Group II
Adrenals	6 30 to 6 62	6 16 to 6 62
Kidneys	6 22 „ 6 59	6 12 „ 6 65

The co-efficients of variability in pH of both these organs is also low (Table III)

It will be noted that in order of increasing acidity the sequence of the organs is the same in the two control groups, except that in Group II the testes and spleen have changed places (Table II)

The Brain of normal pigeons has a mean pH value of 6 8 But it differs, within relatively narrow limits, in different parts of the organ that of the optic lobes and pons being the highest and that of the cerebrum the lowest (Tables II and III) The variability in pH of any part of the brain is normally slight The ranges in pH were as follows —

	Group I	Group II
Optic lobes	6 65 to 7 0	6 72 to 6 98
Pons	6 74 „ 6 91	6 68 „ 6 98
Cerebellum	6 65 „ 6 88	6 65 „ 6 95
Cerebrum	6 55 „ 6 89	6 55 „ 6 86

The Peripheral Nerves, as represented by the sciatics are slightly, though insignificantly, less acid than the brain as a whole The variability in pH is slight (Table III) The range of pH lay between 6 79 and 6 96 in Group I and between 6 74 and 7 0 in Group II

The Muscles are the most acid of the tissues examined those of flight having a higher acidity than those of the lower limbs Their pH is more variable than that of the organs generally, its range was as follows —

	Group I	Group II
Chest muscles	5 51 to 5 95*	5 46 to 5 77
Limb (lower)	5 58 „ 6 12	5 53 „ 6 12

pH of organs and tissues in deficiently-fed birds.

The deficiency common to Diets III, IV and V is one of vitamin B_1 In Diet III this vitamin was destroyed by autoclaving in an alkaline medium, some destruction of vitamin B_2, no doubt, also occurred (Chick and Roscoe, 1930) Autoclaving of the *dhal and bar* in Diet IV had similar effects, but in this diet a small amount of vitamin B_1 was provided by the orange juice Diet V was markedly deficient in all vitamins as well as in protein and mineral elements

The general effect of the deficient diets, considered together, was to cause a significant increase in acidity of the brain, a significant diminution in acidity of the muscles, a slight diminution in acidity of the liver, thyroid and auricle, a tendency to increased acidity of other organs and no appreciable change in the blood But to this generalization there are exceptions

Effect of death on pH —It will be noted from Tables II and III that with few exceptions, and these insignificant, the organs and tissues of deficiently-fed birds that died a natural death were less acid than those of birds killed at the onset of the post mortem† In the former there is a general tendency for the pH values to

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* One abnormally high value (6 96) is excluded

† This reaction is in striking contrast to the increased acidity consequent on post mortem change [illegible] in the organs and tissues removed from healthy animals, and in conformity with the [illegible] alkaline side which occurs in 'fatigued' meat (Brochet, 1932) —R McC

return to a more normal level, though still to remain more acid or more alkaline, as the case might be, than normal Estimations of pH made in birds that have died may, therefore, yield misleading results For this reason Groups III, IV and V are sub-divided into birds that were killed (K) and those that died (D), conclusions as to the changes in hydrogen ion concentration that are brought about by the deficient diets being drawn only from birds killed at the onset of polyneuritis

The Blood —The range of pH was as follows —

Group	III	7 54 to 7 73
„	IV	7 55 „ 7 75
„	V	7 37 „ 7 66

In Groups III and IV the pH of the blood was within normal limits, it was below normal in one of the four cases examined in Group V In this case a pH value of 7 37 was regarded as evidence of acidosis, particularly as it was associated with a corresponding increase in acidity of the organs Gigon (1929) has drawn attention to the absence of acidosis in avitaminosis Our own observations indicate that its absence is the rule, but that exceptional cases do occur in birds fed exclusively on polished rice

The Pericardial Fluid —The number of observations made under this heading are few and admit of no conclusions other than that, next to the blood, the pericardial fluid was the most alkaline of the materials examined

The Heart —The range of pH of the *auricle* and *ventricle*, in birds killed at the onset of polyneuritis, was as follows —

		Auricle	Ventricle.
Group	III	5 95 to 6 89	5 88 to 6 79
„	IV	6 08 „ 6 28	5 66 „ 6 16
„	V	6 14 „ 7 52	5 77 „ 7 17

The mean values (Tables II and III) indicate the significantly greater acidity of the ventricle Of the birds examined in Group III the pH of the auricle was slightly above normal limits in one and below normal limits in another, but in neither to a significant degree, in the remaining five it was within normal limits The ventricle was slightly more acid in two cases, more alkaline in two and normal in the remaining four Of the 4 birds in Group IV in which both parts of the heart were examined, the auricles were slightly more acid in one, and the ventricles more acid in three, than in the corresponding control group (II) In Group V the auricles were more alkaline than normal in two out of 6 cases, the ventricles more acid in two and more alkaline in one.

Amongst the 62 deficiently-fed birds there were three typical and three atypical cases of beri-beri (McCarrison, 1928, Taylor and Thant, 1929) Of these

one was killed at the height of the disease, the others died The individual pH values were as follows —

Number of bird	Died or killed	pH		Difference	Diagnosis
		Auricle	Ventricle		
47	Died	6 24	5 77	0 47	Atypical beri beri
64	,,	6 68	6 24	0 44	,, ,,
89	,	6 79	6 08	0 71	,, ,
86	,,	6 86	5 55	1 31	Typical beri beri
88	,,	6 96	5 95	1 01	,, ,,
101	Killed	6 55	5 77	0 78	,, ,,

It will be noted that in every case the difference in pH between the auricle and ventricle exceeded the normal maximum difference (0 35) and that the excess is greatest in cases of typical beri-beri Unfortunately, five of the six cases of beri-beri died and, in consequence, the issue is obscured by the changes in pH which occur after death though this change may be presumed to effect both parts of the heart equally But in the remaining case the difference between the two parts of the heart is so great as to suggest that a feature of the cardiac condition in true beri-beri may be a disturbance in the electrotonic relation between the auricle and ventricle In no case of polyneuritis (as distinguished from beri-beri), in which the bird was killed at the onset of paralysis, did the difference in pH between the auricle and ventricle exceed the maximum limit to a significant degree The observations here recorded are too few to admit of definite conclusions, but having regard to the cardiac manifestations of true beri-beri they are suggestive

The Thyroid Gland is normally the most alkaline of all organs of the body and the normal range of pH is very limited Our observations in regard to it are relatively few in deficiently-fed birds that were killed But such as they are they indicate no change, so far as the diet of autoclaved grains (III) is concerned In Group IV the pH ranged between 6 54 and 7 49 as compared with a range of from 6 87 to 7 12 in the corresponding control group (II) Only one of the four glands examined in this group (IV) gave a pH value which lay outside normal limits, and in this case there was a definite increase in alkalinity which was associated

with a slight degree of thyroid enlargement In Group V two glands were examined, both were more alkaline than normal, one being a hypertrophic and the other an atrophic organ In this connexion attention may be drawn to the increased alkalinity of the hyperplastic thyroids of rabbits fed exclusively on a diet of fresh, raw cabbage (McCarrison *et al*, 1933)

The Pancreas —Our observations include only one case, killed at the onset of the polyneuritis, in Group III, in this the pH of the pancreas was within normal limits Of the 5 (killed) birds examined in Group IV three yielded pH values less than normal, while two had values within normal limits The tendency, therefore, was for the diet to cause a slight, and insignificant, increase in acidity of the pancreas Two birds fed exclusively on polished rice were killed at the height of the polyneuritis, in both the pH of the pancreas was normal

The Spleen —This organ was examined in six birds of Group III that were killed at the onset of polyneuritis in 4 the pH was lower than normal, in one it was higher, and in the remaining case it was within normal limits Of the 6 birds examined in Group IV all had spleens whose pH values were within normal limits Four birds examined in Group V had spleens whose pH was normal or so nearly so as to be regarded as such

The Testes —These organs were examined in only one bird which was killed at the onset of the polyneuritis (Group IV) The atrophic glands were definitely acid

The Adrenal Glands —The hypertrophic organs were definitely acid in 4 out of 6 cases in Group III, in one out of 6 cases in Group IV, and in 2 out of 5 cases in Group V the remainder having pH values within normal limits or so nearly so as to be regarded as normal

The Brain —Significantly increased acidity of the brain, in all its parts, was the rule in deficiently-fed birds To this rule there was no exception in Groups III and IV, but in Group V there were two cases out of 6 in which the pH of the optic lobes, pons and cerebellum was normal or so nearly so as to be regarded as such, the cerebrum being but slightly on the acid side The other 4 cases in this group conformed to the general rule The range of pH of the different parts of the brain was as follows in the three deficiently-fed groups —

	III	IV	V
Optic lobes	5 84 to 6 59	5 49 to 6 35	6 02 to 7 03
Pons	5 77 „ 6 62	5 77 „ 6 48	6 12 „ 6 82
Cerebellum	5 84 „ 6 59	5 46 „ 6 55	6 28 „ 6 79
Cerebrum	5 57 „ 6 30	5 49 „ 6 33	5 88 „ 6 48

It is significant that when one part of the brain is highly acid the other parts are, as a rule, equally so Similarly, when one part shows little or no departure from normal other parts are affected in a like way Indeed, a high degree of acidity of any organ or an unusual tendency to alkalinity was usually reflected in other organs of the body From these figures, and those given in Tables II and III, it is evident that Diet IV tended to cause greater acidity of the brain than either Diet III or Diet V, the least acidity being produced by the exclusive diet of washed, polished rice (V) Statistically speaking, however, the differences between the three groups are not significant There is a greater degree of variability in pH of the different parts of the brain—as there is in all organs of the body—in the deficiently-fed than in the well-fed birds

The Peripheral Nerves, as represented by the sciatics, were slightly more acid in 3 out of 6 cases examined in Group III, slightly more alkaline in 1, and normal in the other two One out of 5 examined in Group IV was slightly more acid than normal, the other 4 were normal or so nearly so as to be regarded as such Two out of 6 in Group V were more alkaline than normal, the remaining 4 were normal In the one case of true beri-beri which was killed at the height of the disease the pH of the sciatic nerves was normal (6 76), that of the pons and optic lobes was 6 28, that of the cerebellum 6 48, and that of the cerebrum 5 92 The pH of the sciatic nerves was estimated in 17 birds which were killed at the height of polyneuritis In only 4 of these was it less than 6 76—the lowest normal value—and in none of them was this change of significant degree On the other hand, the pH of these nerves exceeded the normal maximum (7 0) in 4 cases out of the 17, but here again the change was not significant The ranges of pH in the 3 deficiently-fed groups were as follows —

	pH of sciatic nerves
Group III	6 52 to 7 17
„ IV	6 52 „ 7 04
„ V	6 76 „ 7 49

The Muscles —An outstanding effect of the deficient diets was a significant diminution in acidity of the muscles pH estimations were made in seven birds that were killed at the onset of the polyneuritis In all but one the pH values, both of the chest and limb muscles were considerably higher than the normal maximum, the range for the muscles of flight lying between 5 77 and 7 20 and for those of the lower limbs between 6 02 and 7 10

Summary.

An account is given of the results of pH determinations in healthy, well-fed pigeons and in pigeons fed on diets of which the dominant deficiency was one of vitamin B_1 The outstanding results of the investigation were the demonstration

of the increased acidity of different parts of the brain, the diminished acidity of the skeletal muscles and liver, the tendency to disturbance of the electrotonic relations of the auricle and ventricle of the heart and the slight tendency to increased acidity of certain organs, in the deficiently-fed birds

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THE NATURE OF THE SO-CALLED 'BLACK SPORES' OF ROSS IN MALARIA-TRANSMITTING MOSQUITOES

BY

LIEUT-COLONEL R KNOWLES, I M S,
Officer-in-Charge,

AND

B C BASU, M SC (CAL),
Entomologist,
Malaria Transmission Inquiry, Indian Research Fund Association
(*From the Department of Protozoology, Calcutta School of Tropical Medicine and Hygiene*)

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INTRODUCTION

IN the course of his classical work on the transmission of malaria by mosquitoes, 1895 to 1899, Sir Ronald Ross came across certain deeply pigmented cyst-like bodies, deep brown or black in colour, in the mid-gut of infected Culex mosquitoes These worried him considerably, as at first he thought that they might be some essential phase in the sporogony cycle of the malaria parasite, or—alternatively—malaria oocysts themselves parasitized by some other protozoon parasite Later, however, he came across the same forms in non-infected mosquitoes, and, although still uncertain as to the true nature of these 'black spores', decided that they constituted no essential part of the sporogony cycle His first mention of them appears to have been in his 'Report on Kala-azar' (Government Press, Calcutta, January 1899), but several references to the matter are given in his 'Memoirs' (Ross, 1923) Drawings of these black spores are given by Ross (1905, 1923), but the first detailed account of them (not accompanied by any illustration) appears to have been that given by Daniels (1900) in reporting on Ross's work to the Royal Society

From that date to this these so-called 'black spores' have constituted a veritable riddle of the Sphinx to malariologists, and many and diverse views as to their nature have been put forward Grassi (1901), in his monograph on malaria, appears to have been the first to publish illustrations of them, whilst the whole subject has been exhaustively reviewed with full illustrations and an extensive bibliography in a recent paper by Bruce Mayne (1929)

During work on the transmission of malaria under different conditions of atmospheric temperature and humidity which has extended over the past year we have not infrequently come across these black spores in infected *Anopheles stephensi*, and an account of our own findings will be given later A study of our own material, a thorough study of Bruce Mayne's paper and of the literature to which he refers, lead us to the conclusion that no less than three, if not four, different structures have been described as 'black spores' or 'chitin corpuscles' by different workers These are —

(*i*) True malaria oocysts which have undergone degeneration, and in which a very heavy deposition of pigment has occurred These, alone, we believe to be the true 'black spores' of Ross

(*ii*) A hyper-chitinization of segment-like portions of the finer ramifications of the tracheal system around the mid-gut and internal viscera, these, we believe, are what Bruce Mayne has chiefly encountered

(*iii*) Fungus infections of the tracheal system

(*iv*) Infections of the mosquito tissues concerned with microsporidian parasites, which are well known to be common parasites of mosquitoes Such infections, with their very refringent spores and polar capsules, however, in no way resemble the true 'black spores'

Bruce Mayne's paper is so exhaustive that its publication here precludes us from attempting a similar historical survey of the literature, as it would be superfluous On the other hand his conclusions are very definite They are as follows —

'(*a*) The 'black spores'' originally described by Ross are not parasites, nor are they any stage in the development of the malaria parasite in the mosquito

'(*b*) The hypothesis of Grassi and other workers that these bodies are concerned with "regression or involution" processes of the oocyst is not confirmed, nor that lowered temperatures favour the production of these bodies

'(*c*) The "black spores" have been found during experiments with avian malaria in uninfected mosquitoes as well as in mosquitoes harbouring the parasites of malaria

'(*d*) In addition, the "black spores" have been observed in the following insects, undeniably unassociated with malaria —

(1) In freshly-emerged, unfed, laboratory-bred females and males of Anopheles and Culex

(2) In house-flies, *Musca* sp

'(*e*) The "black spores" have been found to react to the test for chitin

'(*f*) Most, if not all, "black spores" appear to be chitinogenous thickenings of tracheal tubes This conclusion forms a rational and simple explanation of the various types of the bodies described'

At first sight Bruce Mayne's conclusions seem to be very well founded, but further study of the subject leads us to believe that he has been dealing only with structures (*ii*) above—hyper-chitinization of segment-like portions of the finer ramifications of the tracheal system around the mid-gut and viscera If this be so, then his conclusions apply automatically, but they do not apply to the true 'black spores' of Ross and Grassi

Bruce Mayne's paper is illustrated by four half-tone plates, of which the first two are mainly reproductions of drawings by previous workers of 'black spores', and the last two are photo-micrographs from his own preparations In the letter-press to these illustrations he frequently appends a note showing how the appearance figured fits in with what one may term his tracheal hypothesis On the other hand, we believe that if the three, or four, different types of structures above described have been muddled up together under the title 'black spores', explanations other than those given by Bruce Mayne are possible For convenience' sake we have here reproduced Bruce Mayne's four plates as Plates XXVII and XXVIII of the present paper, photographically reduced in size We may offer brief comments on the different figures concerned

PLATE XXVII

Fig 1 Copied from Ross (1923) Capsule of an empty zygote containing 'black spores' Bruce Mayne remarks, 'These structures are strikingly similar to tracheæ Two are shown with tænidia like lines within dark lined tubular forms, one appears as a continuous unjointed tube with a black core running throughout its length, and an individual rounded body may be interpreted as the visible end of a chitinized tracheal tube' What strikes us about the illustration, however, is the definite but crumpled thin investing membrane of the oocyst around the whole structure It appears to be a wrinkled malaria oocyst containing residual pigmentary masses of tubular form

Fig 2 After Grassi (1901) Brown bodies found unattached in the cœlomic cavity of an anopheline mosquito infected 13 days Bruce Mayne regards these as 'fragments of the tracheæ detached in the process of dissection' We consider, however, that such an appearance would result from the extrusion of pigmented bodies such as those in Fig 1 on rupture of the oocyst on dissection They show no tracheal striation

Fig 3 After Grassi (1901) Capsule of an oocyst of *P falciparum* containing typical 'brown bodies' Bruce Mayne states, 'In some of these forms—(banana like bodies)—one can detect spiral lines very suggestive of portions of tracheal tubes The two rounded bodies may be cross sections of exposed ends of tracheæ which have penetrated the oocyst' What interests us is the presence of a very clearly defined thin investing oocyst membrane whatever the structures are, here they are clearly within the oocyst Tracheal ramifications spread into and enter almost every tissue of the mosquito, but why they should penetrate into the interior of a parasitic oocyst is not clear

Fig 4 After Walch (1922), who 'considers this the complete enveloping of a hypomycete in a heavy chitinous covering on the mosquito's gut The separated forms he refers to as chitinized mycelium' Bruce Mayne remarks, 'I have observed quite a similar appearance in a bifid branched tracheal tube having a transparent envelope formed about a dense chitinous interior' To us, at least, it appears that the structure illustrated in Fig 4 bears no resemblance at all to the black spores of Ross and Grassi as illustrated in Figs 1 and 3 It may be a fungoid infection of the tracheal system

Figs 5 and 6 After Walch (1922) Brown curved bodies from the region of the salivary gland and oocyst on the gut Found by him to be mainly chitinous Bruce Mayne passes no observation We consider that Fig 5 in no way resembles the true 'black spores' as illustrated in Figs 1 and 3

Fig 7 After Ruge (1903) 'A mass of "Ross's spores" on the gut wall of *Culex pipiens* supposed to be developed from sporozoites' Bruce Mayne offers no comment on this figure It is obviously a feltwork of some sort, but whether of mycelium or of hyperchitinized tracheæ it is impossible to say

Fig 8 After Ruge (1903) From gut wall of an infected *Culex pipiens* Bruce Mayne says that the structures are 'distinctly tubular and very suggestive of sections of torn tracheæ' In our opinion they closely resemble the forms in Fig 2, which we interpret as pigmented masses extruded from a ruptured oocyst

Fig 9 After Wenyon (1926)

No 1 Oocyst and black spores in *A maculipennis* infected with *P vivax* Preparation of Colonel S P James

No 2 Isolated 'black spores' from an anopheline Preparation of Colonel S P James

No 3 'Black spores' from a Macedonian mosquito infected with *P falciparum* Preparation by Dr C M Wenyon

With regard to these three Bruce Mayne remarks, 'The general tubular structure in most of the forms shown is noteworthy Their affinity to tracheal tubes is quite suggestive They are said to be probably the result of the death and degeneration of the oocyst contents, the mosquito depositing chitinous material in them' To our mind this is a much more reasonable interpretation than Bruce Mayne's Fig 9 (1) very clearly shows the delicate investing cell membrane of the oocyst, so that the bodies are here certainly within the oocyst, Figs 9 (2) and 9 (3) we interpret as pigmented masses liberated on ruptures of the degenerated oocyst

Fig 10 After Ross (1905) Described as a zygote of the 6th day and later The five forms in the sketch are interpreted as 'black spores' Bruce Mayne remarks, 'The hollow tubular like structure is quite distinct, their resemblance to stranded portions of tracheal tubes is suggestive' Diagrammatic as the drawing is, what interests us is the presence of the clear cut thin investing oocyst membrane, and the situation in which the bodies were found

Fig 11 After Bruce Mayne (1929) 'Photo micrograph Portion of gut wall of *Culex fatigans* A field of the tracheal system showing typical clumping Similar clumps commonly form masses of black spores' With this last statement we entirely disagree

Fig 12 After Brug (1916) 'Thought to be an aggregation of "black spores" formed from the wrinkling of the cyst wall of an oocyst and subsequently becoming chitinized' 'The resemblance to tracheæ is very striking', writes Bruce Mayne This may be the case, but, in our opinion, the resemblance to the true 'black spores' as illustrated in Figs 1, 3 and 9 (1) is so remote as to be unreal

Fig 12*a* After Brug (1916) 'A group of "black spores" or "chitin corpuscles" on the gut wall of a *Culex pipiens* infected with *P præcox* At (*b*) an association of the chitinized structures with the tracheæ is indicated' We can offer no opinion on the structures figured, they have but little resemblance to the true 'black spores' and may be torn elements of tracheæ from a dissection

PLATE XXVIII [After Bruce Mayne (1929)]

In Plate XXVIII we have tried to reproduce in one plate the five very fine photo micrographs and one camera lucida drawing illustrating Bruce Mayne's paper Unfortunately they have suffered somewhat in reduction photographically

Fig 13 'Photo micrograph of mass of "black spores" on gut wall of *Culex fatigans* infected with avian plasmodia These bodies were found to measure actually the same length (10 to 12 5 microns) as the sporozoites expressed from the same specimen of mosquito' (In our opinion this finding would tend to show that the forms depicted are of parasitic rather than of mosquito origin)

Fig 14 'Photo micrograph Gut wall of *Culex fatigans*, 10th day following infective bite Dissected specimen unstained, mounted in formalin There are two free masses of chitin bodies, a group of three in a half grown oocyst, and an oocyst in the segmenting stage measuring 30 microns On the capsule of the latter appears the broken end of a tracheal tube This was found to measure 10 microns, the same as many of the "black spores" shown in the two masses'

This is one of the most interesting of Bruce Mayne's illustrations We confess that we find it difficult to interpret the two large dense masses of chitin in the lower part of the photo micrograph as clusters of 'black spores' The close application of the tracheal tube to the lateral aspect of the oocyst is very clearly shown but it is difficult to agree that its broken off end, measuring 10 microns, resembles a 'black spore'

Fig 15 'Photo micrograph Thoracic muscle close to salivary glands of *Culex fatigans* Here were found three lines of "black spores" and those illustrated give one the impression that they resemble the forms which Walch speculatively regards as "migratory sporozoites which have undergone chitinization while wandering through the body tissue" The black spores figured measured 10 to 15 microns'

It is difficult to interpret the appearances shown The forms depicted certainly suggest parasitic forms working their way along the fascial planes between the muscle fibres On the whole we consider that Walch's 'speculative hypothesis' is that most likely to be correct

Fig 16 'Photo micrograph Thorax of *A subpictus* showing dark brown bodies in and near a large trachea Careful focussing revealed all but four of them surrounded by the tænidial envelope of the trachea These "black spores" measured about 10 microns'

Whatever is shown in Fig 16, it bears but little resemblance to the true 'black spores' as depicted in Plate XXVII, figs 1, 3 and 9 (1)

Fig 17 'Photo micrograph A field on the gut wall of a severely infected *Culex fatigans* shown in the same focus, the dorsal aspect In this view there are 12 typical "black spores", six in the same focus, the remainder lost to view Those clearly shown appear in the mass of tangled tracheæ which are apparently super imposed They seem to have no direct connection with the gut wall tissue, which in other regions is invaded by numerous oocysts, the capsules of which were very prominent On the muscular gut wall of this mosquito, the tracheal system appears unusually well supplied with tracheal tubes which give the impression of concentration in the area selected for illustration'

Whatever the structures figured are, they are certainly chitinous and of tracheal origin We doubt, however, whether they correspond to the true 'black spores' of Ross What appears to be a very deeply pigmented malaria oocyst is shown in the upper left hand part of the illustration

Fig 18 'Camera lucida drawing From the gut wall of an uninfected (wild) *A subpictus* There are shown several branched tracheal tubes merging into tracheoles with a small and a large "black spore", their sizes limited by the varying diameter of the single tracheal tube in which apparently the chitin bodies were deposited In the tube of smaller calibre there were no tænidia visible, whilst the deposition of the chitinous mass at the wider portion seemed to have obliterated the spiral markings of the trachea The larger "black

> spore", measuring 20 microns long by 2 3 microns in width, is typical in form and appearance to the majority of similar bodies observed in the course of these studies'
>
> We admit, at once that Fig 18 very conclusively proves that the structures studied and described by Bruce Mayne are chitinous in character, and almost certainly originate from the chitinogenous cells of the inner lining of the finer tracheal ramifications The question at issue, however, is whether these structures described by Bruce Mayne are or are not the 'black spores' of Ross and the synonymous 'brown bodies' of Grassi In view of the exhaustive character of Bruce Mayne's paper it may seem presumptuous to doubt this, but the evidence which we have to put forward is very strongly opposed to his views

In order to avoid duplicating the review of the previous literature by Bruce Mayne, we have tried to condense the views previously put forward by different workers and analysed by Bruce Mayne in tabular form in Table I, taken for the most part from Bruce Mayne (1929), though the original papers have also been studied in nearly every instance

We believe that a study of Table I and of Plates XXVII and XXVIII will convince any unprejudiced worker that at least two, if not three, different structures of different origin have been included by different workers under the term 'the black spores of Ross'

Our own observations

All, or almost all, previous observers on the subject have described what they have seen in dissections Our work differs from theirs in that we have relied entirely upon section cutting of infected anophelines The study of 'black spores' was not the primary object of our investigation, which was to try to determine the limits of atmospheric temperature and humidity within which malaria-transmission takes place From time to time black spores were observed in these mosquitoes, in each instance rough sketches were made at the time, and the slides marked and laid aside for more detailed study later on Later, these slides were very carefully studied, more accurate drawings made, and photo-micrographs taken The mosquitoes used throughout were female *Anopheles stephensi* Liston, bred out in the laboratory from larvæ collected in Calcutta city from water-tanks, etc, and they were fed upon patients showing the gametocytes of *P vivax*, *P malariæ* and *P falciparum* respectively, as also upon one patient with black-water fever who failed to show malaria parasites

A few notes as to technique may here be given —

Freshly killed mosquitoes were fixed in Bles' fluid After six hours' immersion in the fixative, the wings and legs are cut off with a fine pair of scissors and a small incision is made at the end of the abdomen with a sharp scalpel to facilitate penetration of the fixative

After fixation the body of the insect is washed twice in 70 per cent alcohol, and then brought through the ascending grades of alcohol, one day in each, to absolute alcohol It is then transferred to thin celloidin solution—3 *g* of desiccated celloidin chips in 100 c c of clove oil Ordinarily this mixture takes a month for complete solution, but if the celloidin chips be thoroughly ground into powder in a pestle and mortar before adding to the clove oil, solution is complete within a week

TABLE I

Observations of previous workers upon 'black spores' Summarized from Bruce Mayne (1929)

Observer	Year	Term adopted	Situation and description	Conclusion as to the nature of the forms observed
Ross	1899, 1905, 1923	Black spores	Within oocysts in clusters Also free in tissues Also in uninfected mosquitoes	Not an essential stage in the life cycle of the Plasmodia Possibly 'parasites within parasites'
Daniels	1900	Black spores	Within oocysts, or 'all over the body of the mosquito', originating from ruptured oocysts	May possibly be a phase of the life cycle *via* contamination of water supplies
Ross, Annett and Austen	1900	Black spores	Frequently seen in *A costalis* but never within the capsule of a zygote Often occur mixed with what looked like segments of a fungus within sheaths of muscle fibres and appeared to have no relation at all to the Plasmodium	'It remains to be proved that they have any connection with the parasites of malaria'
Grassi	1901	Brown bodies	Always associated with the presence of sporozoites and oocysts Elongated forms, short rods, and round bodies Either within the oocyst or in a tangled mass with a distinct capsule on the gut wall, the latter a 'residual body' or involution form	Immature or atrophied sporozoites or fragments of sporozoites with a brown envelope formed about them Infestation only occurs during winter and may be associated with lowering of temperature
Ruge	1903	Ross's spores	Stomach and salivary glands of *Culex pipiens* S and comma shaped brownish yellow to blackish brown Black spores and normal sporozoites within same oocyst Transitional forms also seen	Developed from sporozoites
Celli	1904	Black spores of Ross		Probably involution forms of sporozoites

TABLE I—*contd*

Observer	Year	Term adopted	Situation and description	Conclusion as to the nature of the forms observed
Blanchard	1905	Brown bodies	Found only in association with oocysts and derived from sporozoites Distributed in variable numbers from 20 to 50 in a somewhat granular mass	Regression or involution forms of malaria parasites in the mosquito Exist mainly in the hibernating mosquito, being influenced in development by the low temperature
Stephens	1905	Brown spores	In cysts in which sporozoites have disappeared Stresses importance of Italian workers' claim that the brown spores are only found in mosquitoes fed on simple tertian cases	Nothing to do with malaria parasites Due to quite independent infection by some other form of bacterial or protozoon life Vary very much in appearance, so same organism not always present
Stephens and Christophers	1908	Black spores	In or about salivary glands of *A rossi (subpictus)*	Suggestive of a mycelial nature
Bentley	1910	Brown spores	Found in *A stephensi* in Bombay	'Gives definite parasitic value' Compares number of infected stomachs with number showing brown spores—1909 and 1910
Doflein	1911	Ross's spores	Found only in infected mosquitoes	Associated with zygotes and sporozoites in a process of degeneration
Minchin	1912	Black spores		Are degeneration phenomena of malaria oocysts Compares degenerative phenomena in oocysts of *Cyclospora*, *Actinosphærium* and *Amœba proteus* during periods of depression
Neumann, quoted by Prowazek	1912	Black spores	Found in Stegomyia mosquitoes	Black spores are probably degenerated sporozoites Hypertrophied forms of black spores are non motile, distorted sporozoites
Ruge, quoted by Prowazek	1912	Black spores	Especially in mosquitoes fed on naturally infected sparrows	
Hindle	1914	Black spores		Appear to be a species of *Nosema* attacking the mosquito independently of malarial infection

Brug	1916	Chitin corpuscles	One fourth of *C pipiens* infected with *P præcox* showed them on mid gut	Oocyst wall becomes wrinkled and subsequently chitinized Compares chitinized encapsulated microfilariæ Oocyst may rupture during chitiniza tion of its contents
Manson	1917	Black spores		Favours Sambon's view that they are protozoal organisms belonging to the genus *Nosema*, parasitic on the malarial oocyst
Castellani and Chalmers	1919	Black spores	In oocysts on the stomach wall of mos quitoes	Believe them to be protozoan in nature, defining them as hyper parasites of the malaria organism belonging to the genus *Nosema*
Flu	1920	Black spores	In malaria oocysts of human and avian malaria	Invasion of the oocyst by 'black spores' is the result of an over production of chitin on the part of the insect
Thomson and Woodcock	1922	Black or brown spores		Undoubtedly malaria parasites which have degenerated instead of normally completing development Degene rated oocysts may be invaded by other parasites of the mosquito, e g, Microsporidia
Walch	1922	Chitin corpuscles	Yellow to black, yellow brown, or brown and black Not constant in form, varying from spherical and S shaped to banana shaped Observed in non infected as well as in malaria infected anophelines, in thorax, gut and ovaries	Represent parts of the oocysts trans formed into chitin Successive layers of chitin are superimposed thus enshrouding the oocyst Those in thorax, ovaries may be migratory sporozoites which have become chiti nized Those in non infected mos quitoes may be parasites of another protozoan parasite which have become chitinized Quotes instances of invading mycelium in mosquito becoming chitinized
Hegner and Taliaferro	1924	Black spores		Are definitely parasitic 'They ap parently are oocysts parasitized by Microsporidia of the genus *Nosema*'
Ziemann	1924	Black spores of Ross		Have a definite relationship to the malaria parasite Emphasizes views of Brug and Walch on the chitiniza tion of the contents of the oöcyst

TABLE I—*concld*

Observer	Year	Term adopted	Situation and description	Conclusion as to the nature of the forms observed
Manson Bahr	1925	Black spores		Degenerated cell contents of oocysts which have become chitinized
Wenyon	1926	Black spores		Are certainly *not* spores of a microsporidian, as they do not bear any resemblance to these Are probably the result of the death and degeneration of the oocyst contents at various stages of its development Possibly chitinous material is deposited in them by the mosquito
Goodrich	1928	Black spores		Quotes Zwolfer (1926) as stating that they are produced by the action of the insect's phagocytes These secrete pathological chitin which gradually darkens
James	1928	Brown bodies	Scattered through thorax, salivary glands and gut wall in 78 out of 2,300 dissected Black spores are observed invariably in presence of zygotes or sporozoites	Are clearly of malarial origin, inasmuch as the black spores are never found in the absence of malaria parasites nor during period when these forms were undergoing development in the mosquito up to the 7th day
Bruce Mayne	1929	Black spores and rounded bodies	Black spores, 7 to 16μ by 2 5μ Rounded bodies, 5 to 20μ Occur as individuals, in broken lines, small clumps and matted masses Banana, rounded, mass formation and linear types Found in gut-wall, on or in oocysts, thorax tissue, ovaries, along trachea, salivary glands and muscle	Are not parasites Are no stage in the development of the malaria parasite in the mosquito Occur in non infected as well as in infected mosquitoes Also in unfed Anopheles and Culex, and in Musca React to the test for chitin Most, if not all, appear to be chitinogenous thickenings of tracheal tubes

From thin celloidin the insect is transferred to thick celloidin, 8 to 10 per cent, and left there for 12 to 24 hours It is then transferred to a paraffined cover slip and arranged in position The cover slip is next floated on chloroform in a watch glass until the celloidin embedded insect falls off The latter is then transferred to chloroform in a specimen tube—two changes in 24 hours Paraffin is added to the chloroform to saturation for 24 hours, and saturation assured by placing on a water bath at 60°C for an hour or less The final embedding is in paraffin in the ordinary manner Sections are cut at 4 to 5 microns on a sliding microtome, and the ribbons mounted on glass slides coated with Mayer's albumin fixative

Sections were stained for an hour or more in diluted well ripened Delafield's hæmatoxylin, and decolorized in ½ per cent picric acid in 50 per cent alcohol followed by soaking in 70 per cent alcohol until all yellow colour had disappeared from the tissue They were counterstained for two minutes in alcoholic eosin Sections stained by Mallory's chloride of iron hæmatoxylin stain or Heidenhain's iron hæmatoxylin method also give excellent results

Incidence of 'black spores'

In all, nine experimental feeds with different batches of mosquitoes on different patients are concerned Details are given in Table II

It will be seen from Table II that no black spores were seen in connection with *P vivax* infections It will be seen here, however, that the malaria infection rate in the mosquitoes concerned was very low, only 12 out of 141 showing gut infection, or 8 5 per cent In other words, in experiments 1, 2 and 3 we have a low oocyst infection rate associated with an absence of black spores

In experiments 4 and 5 with *P malariæ* the oocyst infection rate is much higher, 38 positive out of 158 sectioned, or 24 0 per cent This is associated with the presence of degenerated and pigmented oocysts, or 'black spores'

In experiments 6, 7 and 8 with *P falciparum*, the oocyst rate is again high, 44 out of 309 or 14 2 per cent or, if only experiments 6 and 7 be considered, 39 out of 77, an oocyst rate of 50 6 per cent Here again the presence of degenerated black oocysts with a high oocyst rate is evident

We have sectioned several normal unfed female *A stephensi* as controls to these fed mosquitoes, chiefly in order to have uninfected controls showing the normal anatomy of uninfected mosquitoes in sections for comparison In none of these have we seen any appearance resembling the 'black spores' of Ross In connection with work on the transmission of dengue, the mid-gut and salivary glands of 366 *Aëdes aëgypti* were examined either by dissection or section-cutting between August 1927 and November 1929 Some of these were adults captured in dengue-infected houses, others were bred out in the laboratory and fed on dengue patients In no instance were black spores encountered Spirochætes, which were especially being looked for, were also not seen, except in one whose gut swarmed with spirochætes

Column 5 of Table II shows that degenerated black oocysts and normal oocysts going on to sporozoite formation are frequently present in the mid-gut of the same mosquito simultaneously

TABLE II

Showing incidence of 'black spores' in A stephensi *fed upon malaria patients*

Number of experiment	Total number of mosquitoes fed and sectioned	Species of malaria parasite concerned	Number of mosquitoes in which black spores were found	Degenerated oocysts plus healthy oocysts in	Gut infection + in	Salivary gland infection + in	Percentage of fed and sectioned mosquitoes showing black spores
Experiment 1	104	*P vivax*	0	0	0	0	0
„ 2	24	*P vivax*	0	0	12	1	0
„ 3	13	*P vivax*	0	0	0	0	0
Experiment 4	70	*P malariæ*	4	1	37	0	5 7
„ 5	88	*P malariæ*	0	0	1	0	0
Experiment 6	30	*P falciparum*	5	2	15	11	16 6
„ 7	47	*P falciparum*	2	2	24	0	4 3
„ 8	232	*P falciparum*	0	0	5	2	0
Experiment 9	24	Black-water fever patient	0	0	0	0	0

We conclude from Table II that the 'black spores of Ross', or at least degenerated and densely pigmented oocysts are found only in mosquitoes in which malaria infection is present

Relationship to atmospheric humidity and temperature

The mosquitoes concerned in these experiments were divided into three main batches (*i*) one batch was kept in an air conditioning cabinet* under Calcutta monsoon conditions for the period from the middle of June to the middle of September, i e, with the wet bulb thermometer constantly at 79 5°F and the dry bulb constantly at 83 5°F, (*ii*) a second batch was kept under Calcutta post-monsoon conditions for the period from the middle of September to the end of November—wet bulb thermometer 73°F, dry bulb 78°F, (*iii*) the third batch was divided between the refrigerator room—38° to 48°F, the 22°C (72°F) cool incubator, the cool room (68° to 84°F), the 27°C (81°F), cool incubator, and room temperature The relative humidities in this third set of experiments varied between 8 and 100 per cent

Results with batches (*i*) and (*ii*) are given in Table III

It will be seen from Table III that the highest incidence of black spores occurred in mosquitoes fed on patients showing the gametocytes of *P falciparum* and kept under monsoon conditions

Details need not be given in tabular form for batch (*iii*) In all 333 *A stephensi* were used, and 112 of them finally sectioned As far as possible the relative humidities were set for each temperature at 8, 15, 35, 55, 75 and 100 per cent—Buxton's method of control of relative humidity by putting the insects in closed desiccators over different strengths of pure sulphuric acid being adopted In no single instance were black spores encountered This method of controlling relative humidity, however, appears to be unfavourable to the development of the malaria sporogony cycle

DESCRIPTION

Plate XXIX shows the appearance of these degenerated and black oocysts as seen in sections of the gut-wall of infected mosquitoes In each instance a rough sketch of the appearances seen was made at the time of finding them, and the slide was kept for future study Later, when the collection was complete, more accurate drawings were made from all the slides concerned, and the 20 figures in Plate XXIX have been selected from a much larger number of drawings as being typical of the

* A description of this cabinet, made by the Air Conditioning Corporation of Calcutta, is given in the Report of the Director in the *Annual Report of the Calcutta School of Tropical Medicine* for the year 1931, p 17

Table III

Incidence of 'black spores' in mosquitoes fed on gametocyte-carriers and kept at Calcutta monsoon and post-monsoon conditions in an air-conditioning cabinet

Number of experiment	Weather conditions	Wet and dry bulb thermometer readings	Relative humidity	Species of malaria parasite concerned	Number of *A stephensi* sectioned	Number showing black spores	Percentage with black spores
Experiment 1	Calcutta monsoon	79 5°F 83 5°F	85 per cent	*P vivax*	104	0	0
„ 2	do	do	do	*P falciparum*	30	5	16 6
„ 3	Calcutta post monsoon	73°F 78°F	77 per cent	*P vivax*	24	0	0
„ 4	do	do	do	*P malariæ*	70	4	5 7
„ 5	do	do	do	*P falciparum*	47	2	4 3
„ 6	do	do	do	Blackwater fever case	24	0	0

The wet and dry bulb temperatures were recorded by a thermo hygrograph inside the air conditioning cabinet The amount of variation in both was less than 1°F

appearances seen Details are given in the letterpress to the plate The figures have been arranged in serial order in order to show what we believe to be the gradual changes in these degenerated and black oocysts

Plate XXX consists of a set of untouched photo-micrographs from the same set of slides These are selected as those which show the changes most clearly and as being those most suitable for photographic reproduction from amongst a much larger series

These degenerated oocysts were seen only in the mid-gut of infected mosquitoes, and chiefly towards the hinder end of the mid-gut Most of them were found embedded within the epithelial layer of the mid-gut, a few were found partially bulging out into the hæmatocœlic cavity

In a single mosquito there may be found —

(*i*) Only a few completely degenerated oocysts towards the hinder end of the mid-gut

(*ii*) Partially degenerated oocysts, with or without (*i*)

(*iii*) Findings (*i*) or (*ii*) associated with perfectly healthy oocysts showing sporozoite formation

The number of these degenerated oocysts encountered in a single mosquito varied from 1 to 16 Twenty-seven of these degenerated oocysts were measured , their diameter varied from 9 to 16 2 microns, whereas that of the normally developed oocyst of medium development was from 10 to 40 microns The degenerated oocysts are therefore smaller than normal

Plates XXIX and XXX show what we believe to be the process of degeneration and pigmentation in these oocysts Throughout the whole process it will be seen that the delicate investing cell membrane of the oocyst is retained intact It appears to us clear from Plates XXIX and XXX that the pigment is derived from the parasite and not from the mosquito The pigment is at first laid down in the form of small chunks and rounded bodies As these coalesce and more and more pigment is laid down the pigmented masses within the oocyst tend to show banana-shaped, comma-shaped, and oval-shaped forms Finally, when the oocyst has completely degenerated, it is a rounded to oval structure, still showing the delicate investing cell membrane of the oocyst, but completely filled with black pigment The pigment throughout, in stained sections, is brown-black to jet-black in colour Cysts at all stages of degeneration and pigmentation may be found, as illustrated in Plates XXIX and XXX

The characteristic shapes of these pigmented masses remains to be explained It will be remembered that in the development of the malaria oocyst no true sporoblasts or sporocysts are formed Instead there is an increasing vacuolation of the cytoplasm of the oocyst until its internal structure becomes sponge-like in

character As the sporozoites form they are produced in faggot-like bundles It seems likely that this arrangement in faggot-like bundles may be due to the formation within the oocyst of very delicate septa breaking up the interior into loculi, within each of which loculi a bundle of sporozoites lying parallel is produced Should the pigmentary degeneration be confined to one such loculus, or one such bundle of sporozoites, the banana-shaped, sausage-shaped, and rounded pigmentary masses would be accounted for

'Black spores' lying free in the cœlomic cavity, or in the thoracic muscle or around the salivary glands or elsewhere—such as are illustrated in Plate XXVII, figs 2 and 9 (2), (3)—we believe to be the result of rupture of degenerated and pigmented oocysts in dissection Indeed such rupture of degenerated and pigmented oocysts might occur in Nature and this would scatter the pigmented masses into the cœlomic cavity and elsewhere

Although we have at present seen no evidence of chitin in our sections, it seems likely that degenerated oocysts and degenerated pigmentary masses set free into the tissues of the mosquito might later act as foreign bodies and become chitinized Thus Walch (1922) quotes Fulleborn as having observed a filarial larva surrounded by a chitinous envelope in the thoracic muscles of a mosquito, and compares this process to chitinization of the 'black spores' Manson-Bahr (1925, p 514) figures such a chitinized filarial larva It appears to be the rule in insects that foreign bodies or dead parasites within the insect's tissues become invested with layers of chitin laid down on them by the chitinogenous cells lining the inner surface of the fine tracheal ramifications

Degeneration among the Protozoa is a subject which has been too little studied According to Minchin (1912) 'From a consideration of the various examples of degeneration from different causes, it appears that the first part to be affected is always the nucleus, and that the other derangements of the structure and functions of the body are secondary consequences of an abnormal condition of the nucleus'

We have searched such literature as is available to us with regard to the phenomenon of pigmentary degeneration among the Protozoa, but the only author who appears to deal with the subject is Minchin (1912) The hæmozoin of malaria parasites is presumably of the nature of a 'plastid' substance elaborated by nuclear activity and laid down in the cytoplasm It is not essential to all phases of the parasite's life for it is absent from the merozoites, the sporozoites and the early young ring-trophozoites, presumably it is an excretion rather than a secretion, especially as it is ejected after rupture of the mature schizont-rosette Under conditions of failure to develop, it seems not unlikely that breaking down of the chromatin with deposition of enormous quantities of pigment within the oocyst will account for the appearances seen in the 'black spores of Ross' Minchin

quotes by way of analogy similar degeneration-phenomena observed by Schaudinn (1902) in the oocysts of *Cyclospora caryolytica*, and the transformation of chromidia into pigment in the degeneration of *Actinosphærium* described by Hertwig (1904)

SUMMARY

We believe that the confusion which at present exists in the literature with regard to the ' black spores of Ross ' is due to the fact that different observers have been dealing with three different structures under that name, viz —

(*i*) Degenerated and hyper-pigmented malaria oocysts, which may be either intact with the investing thin oocyst membrane unruptured, or with the scattered contents of the same after rupture of the oocyst Such structures may very possibly act as foreign bodies in the tissues of the mosquito and subsequently become chitinized These, alone, we believe to be the true ' black spores of Ross '

(*ii*) ' Chitin corpuscles ' which consist of hyper-chitinization of localized portions of the finer ramifications of the tracheal system We believe that it is on a study of these forms alone that Bruce Mayne (1929) has come to the conclusion that the ' black spores ' are of purely tracheal origin and have nothing to do with the malaria parasites

(*iii*) Fungus infections of the tracheal system of the mosquito, possibly in places associated with hyper-chitinization

Our own observations, based upon material studied in sections, lead us to the belief that (*ii*) and (*iii*) have nothing to do with the true ' black spores '

An account is given of the occurrence of ' black spores ' in infected *Anopheles stephensi* after feeding on patients showing infections with *P. malariæ* and *P falciparum* The process of evolution of these degenerating and hyper-pigmented cysts is described and illustrated

The term ' black spores ', although of historical interest, may be rather confusing, and perhaps ' degenerated and hyper-pigmented oocysts ', or ruptured contents of the same should be substituted

ACKNOWLEDGMENTS

We have to acknowledge most gratefully the constant help, advice and direction given to us by Lieut.-Colonel H W Acton, C I E , I M S , Director, Calcutta School of Tropical Medicine, during the course of this work Our special thanks are also due to Mr H M Roy, artist of the Calcutta School of Tropical Medicine, for the execution of Plate XXIX over which he has taken endless trouble This plate was constructed by drawing from the original slides, with the previous rough sketches at his side We are also very much indebted

to Mr U K Nag, artist of the Calcutta School of Tropical Medicine, for the photo-micrographs in Plates XXVII, XXVIII and XXX

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Explanation of Plate XXVII

Reduced photographic reproduction of Plates IX and X of the paper by Bruce Mayne (1929), showing illustrations of ' black spores ' by previous workers

(For details, *see* text)

Fig 1 After Ross (1923), showing the capsule of an empty zygote containing ' black spores '

,, 2 After Grassi (1901) Brown bodies found unattached in the cœlomic cavity of an infected anopheline mosquito

,, 3 After Grassi (1901) Capsule of an oocyst of *P falciparum*, 11th day of incubation, containing typical ' brown bodies '

,, 4 After Walch (1922) He considers this the complete enveloping of a hyphomycete in a heavy chitinous covering on the mosquito's gut

Figs 5 and 6 After Walch (1922) Brown curved bodies found in the region of the salivary gland and of oocyst on gut

Fig 7 After Ruge (1903) A mass of ' Ross's spores ' on the gut-wall of *Culex pipiens* supposed to be developed from sporozoites

,, 8 After Ruge (1903) Found on the gut-wall of an infected *Culex pipiens*, regarded by the author as transition forms between brown sporozoites and Ross's ' black spores '

,, 9 After Wenyon (1926)

No 1 Oocyst and ' black spores ' in *A maculipennis* infected with *P vivax* (Preparation by Colonel S P James)

No 2 Isolated ' black spores ' from an anopheline (Preparation by Colonel S P James)

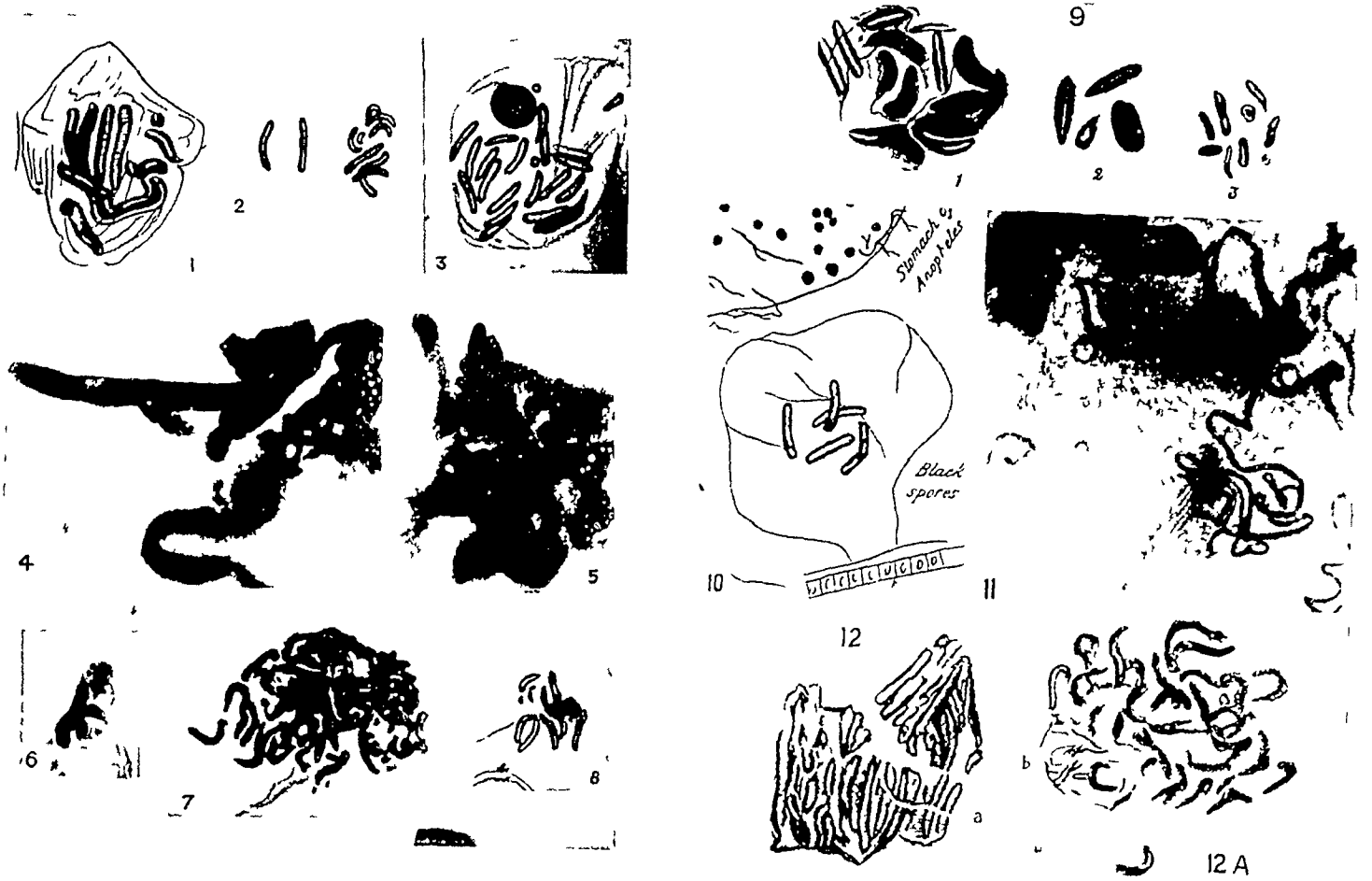
No 3 ' Black spores ' from a Macedonian mosquito infected with *P falciparum* (Preparation by Dr C M Wenyon)

,, 10 After Ross (1905) Described as a zygote of the 6th day and later The five forms in the sketch are interpreted as ' black spores '

,, 11 After Bruce Mayne (1929) ' Photo-micrograph of portion of gut-wall of *Culex fatigans* A field of the tracheal system showing typical clumping '

,, 12 After Brug (1916) Thought to be an aggregation of ' black spores ' formed from the wrinkling of the cyst-wall of an oocyst, and subsequently becoming chitinized

,, 12A After Brug (1916) A group of ' black spores '—' chitin corpuscles ' on the gut-wall of a *Culex pipiens* infected with *P præcox*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2
3
4
5
6
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1
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Stomach of Anopheles
Black spores
10
11
12
a
b
12 A

Explanation of Plate XXVIII

Reduced photographic reproduction of Plates XI and XII of the paper by Bruce Mayne (1929)

(For details, *see* text)

Fig 13 Photo-micrograph of mass of ' black spores ' on gut-wall of *Culex fatigans* infected with avian plasmodia

„ 14 Photo-micrograph of gut-wall of *Culex fatigans* 10th day following infective bite Dissected specimen unstained, mounted in formalin There are two free masses of chitin-bodies, a group of three in a half-grown oocyst and an oocyst in the segmenting stage

„ 15 Photo-micrograph of thoracic muscle close to salivary glands of *Culex fatigans* Here were found three lines of ' black spores '

„ 16 Photo-micrograph of thorax of *Anopheles subpictus* showing dark brown bodies in and near a large trachea

„ 17 Photo-micrograph of field on the gut-wall of a severely infected *Culex fatigans* shown in the same focus, the dorsal aspect In this view there are 12 typical ' black spores ', six in the same focus, the remainder lost to view

„ 18 Camera lucida drawing From the gut-wall of an uninfected (wild) *Anopheles subpictus* There are shown several branched tracheal tubes merging into tracheoles with a small and a large ' black spore '

Plate XXVIII

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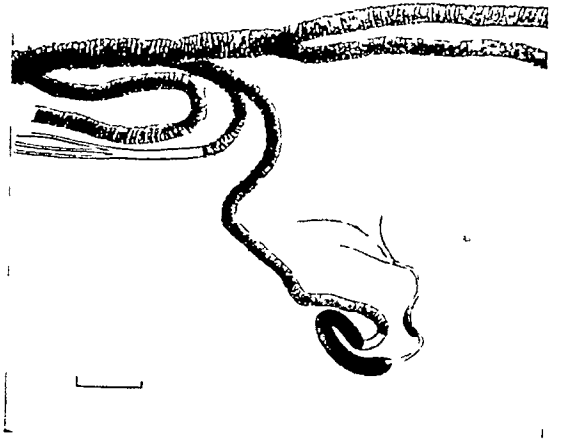

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Explanation of Plate XXIX

(Original)

Drawings from sections of laboratory bred ♀ *Anopheles stephensi* fed upon gametocyte carriers of *P malariæ* and *P falciparum* Showing phases of pigmentary degeneration in malaria oocysts

Fig 1 Fed 19 days previously on a crescent carrier, and kept at monsoon conditions Showing a degenerated oocyst in the epithelial layer of the middle part of the mid gut, located towards the cœlomic cavity Black in colour, diameter 14 4μ

„ 2 Fed 23 days previously on a crescent carrier and kept at monsoon conditions Showing a partially degenerated oocyst in the epithelial layer of the hind part of the mid gut Brown in colour, diameter 14 4μ

„ 3 Fed 25 days previously on a crescent carrier and kept at monsoon conditions Showing a partially degenerated oocyst in the epithelial layer of the middle part of the mid gut Black in colour, diameter 13 3μ

„ 4 Fed 23 days previously on a crescent carrier and kept at monsoon conditions Showing a partially degenerated oocyst in the epithelial layer of the hind part of the mid gut Black in colour, 13 5μ in diameter

„ 5 Fed 23 days previously on a crescent carrier and kept at monsoon conditions Showing a partially degenerated oocyst in the epithelial layer of the hind part of the mid gut Deep brown in colour, diameter 9 1μ

„ 6 Fed 8 days previously on a gametocyte carrier of *P malariæ* and kept at post monsoon conditions Showing a degenerated oocyst in the epithelial layer of the middle portion of the mid gut, located towards the lumen of the gut Black in colour, 12 7μ in diameter

„ 7 Fed 23 days previously on a crescent carrier and kept at monsoon conditions Showing a partially degenerated oocyst in the epithelial layer of the hind part of the mid gut Black in colour, 9 1μ in diameter

„ 8 Fed 8 days previously on a gametocyte carrier of *P malariæ* and kept at post monsoon conditions Showing a degenerated oocyst in the epithelial layer of the middle portion of the mid gut Deep brown in colour, 14 4μ in diameter

„ 9 Fed 25 days previously on a crescent carrier and kept at monsoon conditions Showing a degenerated oocyst in the epithelial layer of the middle portion of the mid gut Black in colour, 14 4μ in diameter

„ 10 Fed 25 days previously on a crescent carrier and kept at monsoon conditions Showing a degenerated oocyst in the epithelial layer of the middle portion of the mid gut Black in colour, 13 5μ in diameter

„ 11 Fed 23 days previously on a crescent carrier and kept at monsoon conditions Showing a degenerated oocyst in the epithelial layer of the hind part of the mid gut Black in colour, 14 4μ in diameter

„ 12 Fed 25 days previously on a crescent carrier and kept at monsoon conditions Showing a degenerated oocyst in the epithelial layer of the front part of the mid gut Jet black in colour, 13 5μ in diameter

„ 13 Fed 23 days previously on a crescent carrier and kept at monsoon conditions Showing a degenerated oocyst in the epithelial layer of the hind portion of the mid gut Deep brown in colour, 9 1μ in diameter

„ 14 Fed 8 days previously on a gametocyte carrier of *P malariæ* and kept at post monsoon conditions Showing a degenerated oocyst in the epithelial layer of the hind part of the mid gut Black in colour, 13 5μ in diameter

„ 15 Fed 23 days previously on a crescent carrier and kept at monsoon conditions Showing an almost completely degenerated oocyst in the epithelial layer of the hind part of the mid gut Jet black in colour, 13 5μ in diameter

„ 16 Fed 8 days previously on a gametocyte carrier of *P malariæ* and kept at post monsoon conditions Showing an almost completely degenerated oocyst in the epithelial layer of the hind portion of the mid gut Black in colour, 11 7μ in diameter

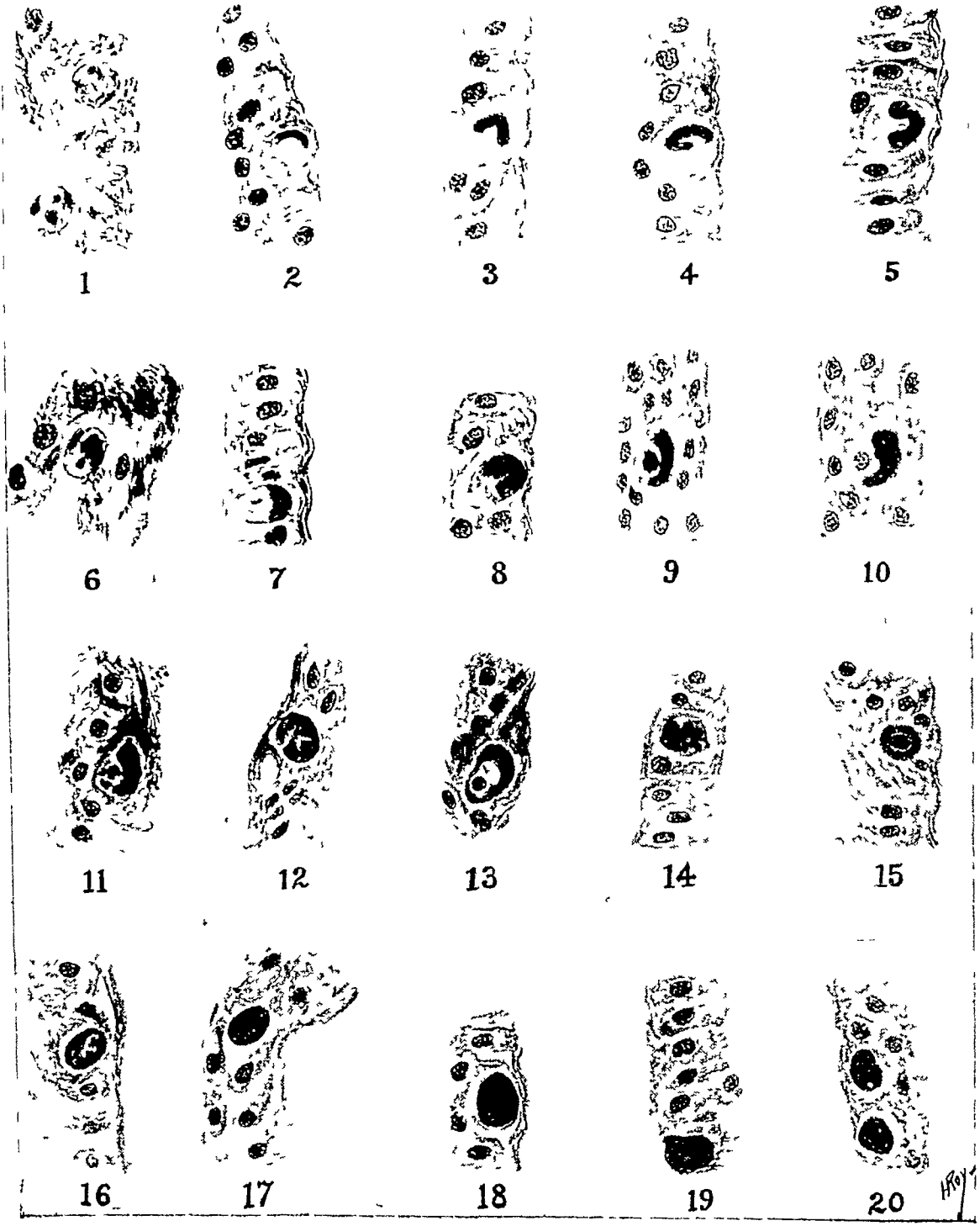
„ 17 Fed 8 days previously on a gametocyte carrier of *P malariæ* and kept at post monsoon conditions Showing a completely degenerated oocyst in the epithelial layer of the hind portion of the mid gut Deep brown in colour, 9 9μ in diameter

„ 18 Fed 23 days previously on a crescent carrier and kept at monsoon conditions Showing a completely degenerated oocyst in the epithelial layer of the hind portion of the mid gut Jet black in colour, 9 9μ in diameter

„ 19 Fed 23 days previously on a crescent carrier and kept at monsoon conditions Showing a completely degenerated oocyst in the epithelial layer of the hind portion of the mid gut Deep brown in colour, 14 4μ in diameter

„ 20 Fed 23 days previously on a crescent carrier and kept at monsoon conditions Showing two completely degenerated oocysts in the epithelial layer of the hind portion of the mid-gut Jet black in colour, 14 4μ and 13 5μ in diameter

PLATE XXIX



Explanation of Plate XXX

(Original)

Photo-micrographs of sections of laboratory-bred ♀ *Anopheles stephensi* fed previously on gametocyte-carriers of *P malariæ* and *P falciparum* 1-12th inch oil-immersion objective, No 2 ocular

The arrows point to the degenerated and pigmented oocysts

Fig 1 Fed 19 days previously on a crescent-carrier and kept at monsoon conditions Showing a partially degenerated oocyst in the epithelial layer of the middle portion of the mid-gut, located towards the hæmatocœle Black in colour, 14 4μ in diameter

„ 2 Fed 8 days previously on a gametocyte-carrier of *P malariæ* and kept at post-monsoon conditions Showing a partially embedded oocyst embedded in the epithelial layer of the middle portion of the mid-gut Deep brown in colour, 14 4μ in diameter

„ 3 Fed 8 days previously on a gametocyte-carrier of *P malariæ* and kept at post-monsoon conditions Showing a degenerated oocyst embedded in the epithelial layer of the middle portion of the mid-gut, located towards the lumen of the gut Black in colour, 12 7μ in diameter

„ 4 Fed 8 days previously on a gametocyte-carrier of *P malariæ* and kept at post-monsoon conditions Showing a degenerated oocyst embedded in the epithelial layer of the middle portion of the mid-gut Black in colour, 14 4μ in diameter

„ 5 Fed 25 days previously on a crescent-carrier and kept at monsoon conditions Showing an almost completely degenerated oocyst embedded in the epithelial layer of the middle portion of the mid-gut Black in colour, 12 6μ in diameter

„ 6 Fed 8 days previously on a gametocyte-carrier of *P malariæ* and kept at post-monsoon conditions Showing a completely degenerated oocyst in the epithelial layer of the hind part of the mid-gut Jet black in colour, 9 9μ in diameter

„ 7 Fed 23 days previously on a crescent-carrier and kept at monsoon conditions Showing a completely degenerated oocyst in the epithelial layer of the hind part of the mid-gut Jet black in colour, 10μ in diameter

„ 8 Fed 8 days previously on a gametocyte-carrier of *P malariæ* and kept at post-monsoon conditions Showing a completely degenerated oocyst in the epithelial layer of the middle portion of the mid-gut Deep brown in colour, 16 2μ in diameter

„ 9 Fed 23 days previously on a crescent-carrier and kept at monsoon conditions Showing two completely degenerated oocysts embedded in the epithelial layer of the hind part of the mid-gut Jet black in colour, 14 4 and 13μ in diameter

PLATE XXX

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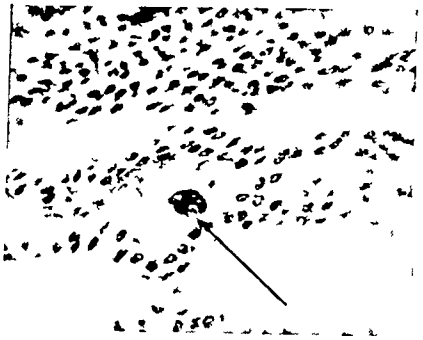

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Ind Jour Med Res, XX, 3, *January*, 1933

THE PHARMACOLOGICAL ACTION OF *PLUMBAGO ZEYLANICA* AND ITS ACTIVE PRINCIPLE (PLUMBAGIN)

PLUMBAGO ZEYLANICA LINN H F B I III 480—ROX B 155-N-O PLUMBAGINEÆ

BY

B B BHATIA, MD, MRCP (London),

AND

S LAL, MB, BS

(*From the Department of Pharmacology, King George's Medical College, Lucknow*)

[Received for publication, July 12, 1932]

VERNACULAR NAMES

WHITE lead wort (English), Ceylonische Bleiwurz (German), Dentelaire de ceylon (French), Agni-Shikha, Chitraka, also Vahni and all other names of Fire (Sanskrit), Chitra, Chita, Chitraka, Lalchitra (Hindi), Chitu (Bengali), Chatramul, Chitrakmul (Dec), Venchittira (Tamil), Tella-chitra (Telugo), Kotuveli or Vellakotu-Veri (Mal), Sudunitul (Sinhalese), Shitaraj (Arabic), Shitaruk (Persian), Ku-Khenphia (Burman), Bile-Chitramul (Can)

Botanical characters—It is an ever-green perennial herb, growing wild or cultivated in gardens, throughout India, with several cylindric, glabrous, striate, stems, alternate exstipulate, entire, sessile leaves $1\frac{1}{2}$ to $3\frac{1}{2}$ inches long and white scentless regular bi-sexual flowers on long spike-like racemes Roots are long, succulent, often much contorted Procumbent branches which on reaching the soil often strike roots

Habitat—Can be cultivated Grows wild almost throughout India Common in Bengal, South India and Kumaun hills

Parts used—The root

Chemistry—Chemistry of *Plumbago* was worked as early as 1883 by Dulong who extracted, from *P europea*, an active principle and called it plumbagin Later

in 1887 Fluckiger isolated the same substance in a purer form, from the roots of *P zeylanica* In 1888 Bettink gave plumbagin the provisional formula of $C_{16}H_{13}O_6$ No further work on the plant or its neutral principle is traceable till Roy and Dutt (1928) of the Allahabad University Chemical Laboratory worked out a simple method of isolating plumbagin in pure form and attempted to solve its constitutional formula It is to these workers that we are indebted for supplying the active principle which enabled us to start the following pharmacological investigation

Plumbagin is a neutral substance in the form of fine glistening silky needles of golden yellow colour With ferric chloride it gives a red colour and dissolves to a cherry red solution in alkalis It is freely soluble in ether, chloroform, alcohol, acetone, benzene, acetic acid, etc Moderately soluble in hot water and almost insoluble in cold water On oxidation by neutral or alkaline potassium permanganate in the cold, plumbagin yields benzoic and cinnamic acids, whereas by distillation with zinc-dust it produces naphthalene and *B*-methyl-naphthalene These facts, together with its yellow colour and penetrating quinone-like smell, led Roy and Dutt (*loc cit*) to think that plumbagin might be a derivative of *p*-benzoquinone of *a*-naphthoquinone with formula of $C_{18}H_{15}O_5$ They further found that plumbagin is present in all the three varieties of plants, viz, *P zeylanica, P rosea* and *P europea* in varying proportions with a maximum of 0 9—1 per cent in some species of *P zeylanica* and *P rosea* *P europea* has much less plumbagin content The exact proportion varies within wide limits according to season, soil, locality, growth, etc In general it is found that the bigger the plant and the drier the soil, the greater is the quantity of plumbagin found in its roots, further the fresher the roots, the greater the proportion of active principle which seems to get oxidized with storage No plumbagin is contained in the aerial parts of the plant with the exception of a small quantity in that part of stem which is near the roots (T E Gerock)

History and alleged uses

In Chitraka these plants are described as digestive, astringent, hot and appetizing A remedy for piles, dyspepsia, leprosy, anasarca, worms, cough, phlegm, flatulence and biliousness In the Nighantas, among other synonyms, they bear the names, Daruna, Dahans, and Agni, in allusion to their burning and acrid properties It also enters into the composition of several preparations used as caustics Locally it is recommended in paralysis and rheumatism Mohammedan writers, who treat the drug under the name of Shitaraj, describe it as caustic, vesicant, expellant of phlegmatic humours, digestive, anti-rheumatic and abortifacient Mir Mohammad Hussain speaks of several kinds of Shitaraj and says one of them is the hhadhyum or hfadhyum of the Greeks Sprengel considers τριπολιον of Dioscorides to be *P europea* Dr Oswald used it in the treatment of

intermittent fever and thought it to be a powerful sudorific Ainslee speaking of *P rosea* describes it as acrid and stimulating, useful in rheumatism and paralytic affections O'Shaughnessy after several trials expressed favourable opinion about its being a cheap substitute as a vesicant for cantharides Waring found its blisters to be much more painful than those of ordinary vesicants R Gray claims to have cured certain cases of leucoderma with it Dr Bhattacharjee found it useful in post-partum hæmorrhage It is frequently used to procure criminal abortion and occasionally for suicidal purposes In Japan, from very early times, the drug has been used for the treatment of a number of complaints such as neuralgia, rheumatism, toothache, bald-headedness, ulcer, scabies, carcinoma, gastro-enteric diseases, hæmorrhoids, intermittent fever, syphilis, viper's bite, etc , and as a sudorific, diuretic, emmenagogue and ecbolic (Kein and Ko, 1931)

It is obvious from the varied uses to which this drug has been put that the practitioners of indigenous systems of medicine attached a great deal of importance to it Originators of these systems were as a rule shrewd observers and it is surprising that they could honestly believe that one single drug could possibly be employed for relief of as many as fifteen different ailments We started work on the drug with the object of finding out if this drug had any potent active principle which could be utilized for rational treatment of any of the large number of ailments which it is supposed to cure

Pharmacology

(1) *Procedure* —All experiments were carried out on healthy dogs, rabbits, guinea-pigs and frogs Morphine-urethane anæsthesia was invariably used in doses of 5 mg of morphine and 1 8 grammes of urethane per kilogram of body-weight The anæsthesia induced was almost always deep and even, and rarely required to be supplemented by open ether The following preparations were used —

(*i*) plumbagin, obtained from the chemical laboratory of the Allahabad University,

(*ii*) freshly prepared decoction from the powdered roots (one year old) strength 1 in 10,

(*iii*) freshly prepared tincture from one year old roots, strength 1 in 10, concentrated on water bath just before use and diluted with saline or Ringer's solution,

(*iv*) all injections were made at body-temperature,

(*v*) doses, unless otherwise stated, are per kilogram of body-weight

(2) *External actions* —(*a*) Germicidal value plumbagin, as 1 in 10,000 solution in broth, prevents the growth of *Bacillus typhosus*, after 24 hours incubation High dilutions up to 1 in 50,000 inhibit the growth of *Bacillus typhosus*

(*b*) Toxicity A 1 in 50,000 solution of plumbagin kills *Paramœcium caudatum* after a contact of 2 hours and 1 in 20,000 solution after a contact of half an hour

Frogs, receiving 0 1 mg per gramme of body-weight through their lymph sacs, showed slight stimulation followed by drowsiness and death in 10 to 12 hours

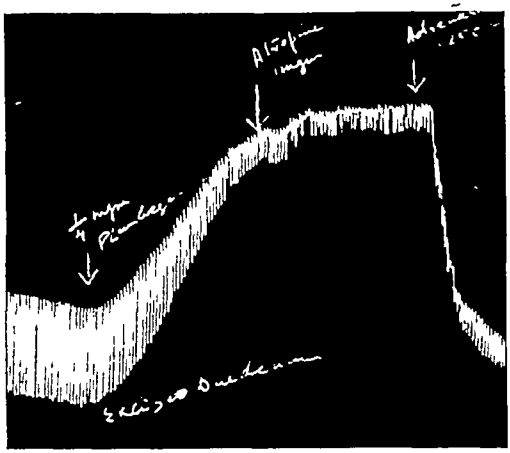
Rabbits receiving subcutaneous injection of 2-3 mg per kilo weight showed no marked toxic effects, except slight loss of body-weight, which was soon recouped

(*c*) Local action plumbagin solution, in all dilutions, stains the skin and mucous membranes yellow—the mark, however, soon changes to a blackish colour and then persists for several days or even months Solution of plumbagin, when applied on skin in 1 in 1,000 strength, produces erythema, higher strengths cause blisters, which are very painful Even very dilute solutions irritate mucous membranes Subcutaneous injections cause inflammation rapidly leading to sloughing Taste is acrid and pungent

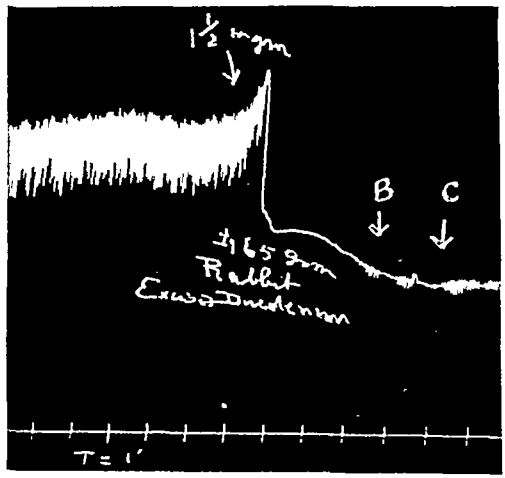
Alimentary system—Rabbits, receiving *per os* a daily dose of 1–2 c c of decoction or $\frac{1}{4}$–1 mg of plumbagin, showed increased appetite and gain in weight, with higher doses 10–15 c c of decoction or 3–5 mg of plumbagin there was lethargy, loss of appetite and diminution in weight *In situ*, the normal peristalsis of the ileum of dog (Finger-cot method) increased in intensity and frequency with $\frac{1}{4}$–1 mg doses of plumbagin or $\frac{1}{4}$–$\frac{1}{2}$ c c doses of decoction and tincture The action was present though less marked after severance of the vagi and atropinization of their nerve endings (Graphs *d*, *e* and *f*) In the excised duodenum of rabbit, ileum of guinea-pig, and stomach of frog, the tone increases and amplitude of contractions diminishes, in dilutions of 1 in 800,000 to 1 in 200,000 of plumbagin or 1 in 200 to 1 in 100 of decoction This increase in tone was not abolished with atropine but was reduced by adrenaline (Graph *a*) With higher doses of plumbagin there was relaxation followed by paralysis (Graph *b*) At this stage, if the drug was immediately removed by repeated washing with Ringer-Locke solution, the rhythmic movements returned, but if the drug was left in contact for a few minutes then no response was obtained with such drugs as barium or pilocarpine Similar results though gradual in onset were obtained from intestinal strips, after their vagal nerve-endings were atropinized (Graph *c*)

Circulatory system—The blood-pressure in dogs rises with $\frac{1}{4}$–$2\frac{1}{2}$ mg doses of plumbagin but the rise is very transitory and is followed by fall Successive doses produced similar effect Large single dose of 5 mg produced only a fall in blood-pressure These effects appeared to be independent of vagus control, as similar results were obtained after the vagi were cut and their nerve-endings atropinized Neither did these effects appear to be related to the sympathetic nervous system,

as no change was observed after paralysis with nicotine and ergotoxin (Graphs *d*, *e* and *f*) Exactly similar results were obtained with decoction and tincture in ½–1 c c doses Perfusion of dog's leg, through the femoral artery with 1



a



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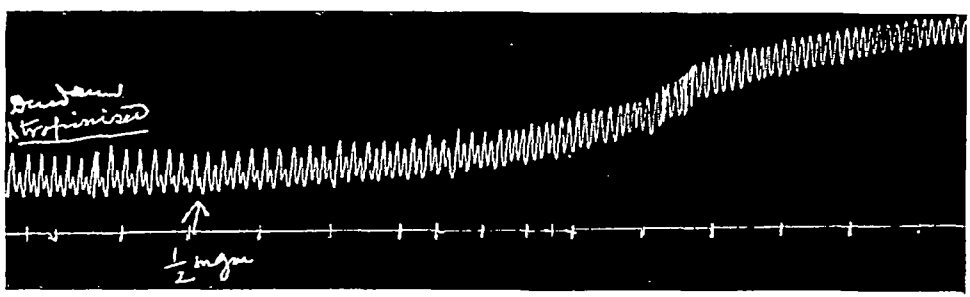
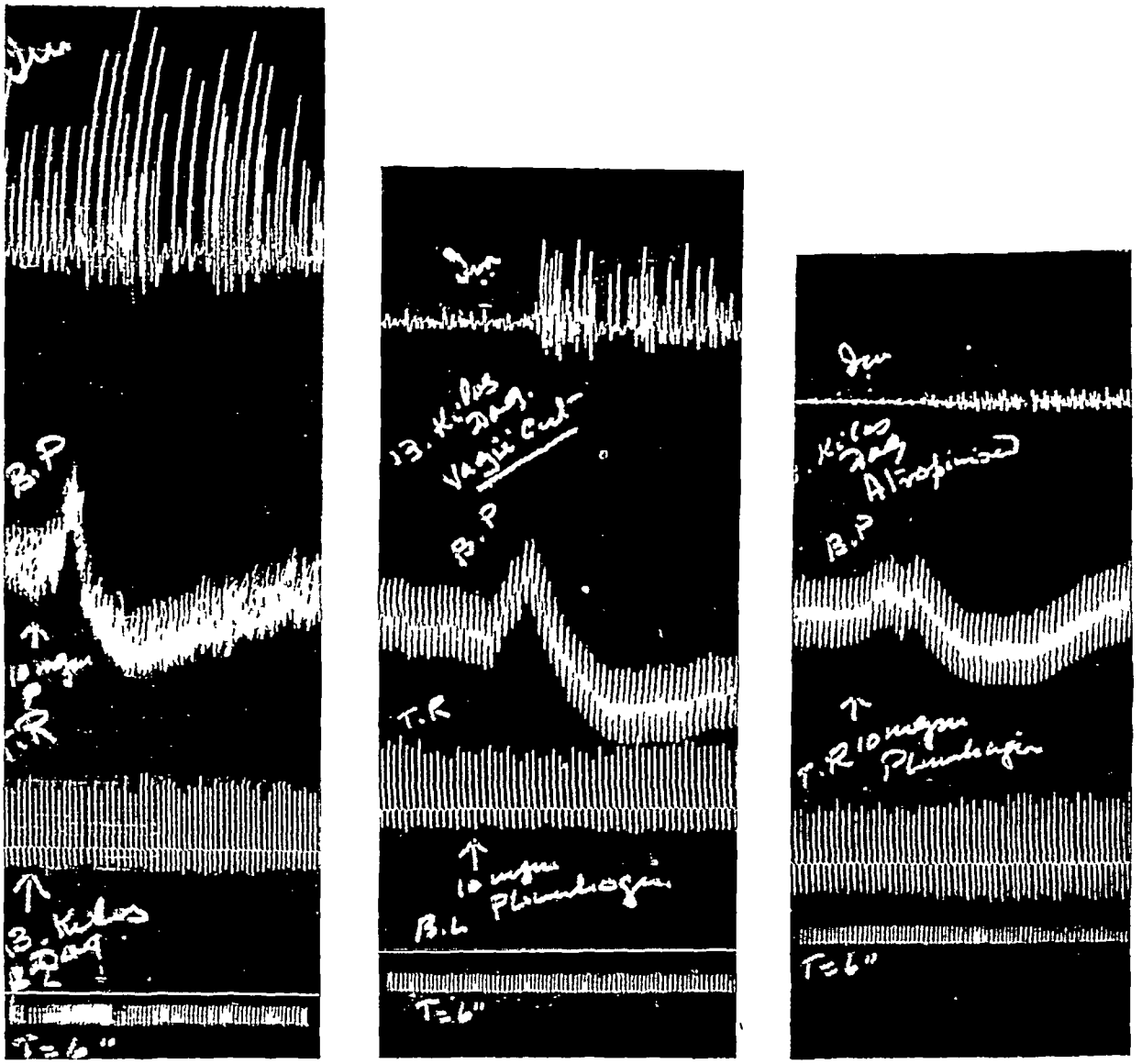


c

Graphs *a*, *b* and *c* —465 grammes Rabbit—excised strip of duodenum suspended in 200 c c of Locke's solution, upstroke=contraction Time, in one minute Graph *a* shows marked increase in tone, on adding ¼ mg of plumbagin to the bath, uneffected by 1 mg of atropin sulphate but relaxation followed 0 25 c c of 1 in 1,000 adrenalin solution Graph *b* shows the effect of 1 mg of plumbagin on another strip of duodenum from the same animal, suspended in 200 c c of Locke s solution At B and C bath changed, with return of rhythmic movements, which later on attained their normal size and tone Graph *c* shows the effect of ½ mg after the nerve endings were efficiently atropinized

in 100,000 solution of plumbagin, increased the outflow through the femoral vein from 48 c c to 52 c c per minute Perfusion of the hind leg of frogs (Loewen-Trendelenburg's method) with 1 in 100,000 solution of plumbagin

increased the outflow, as recorded by a drop counter (Graph *g*) Perfusion of blood-vessels of an excised kidney of sheep also showed similar results Fall in blood-pressure may therefore be partly a vaso-dilator effect



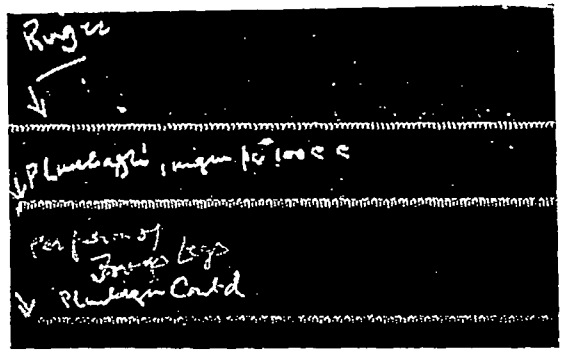
(*d*) (*e*) (*f*)

Graphs *d*, *e* and *f*—13 kilos, male dog Morphine—urethane anæsthesia From above downwards —ileum (Finger cot method) Blood pressure, carotid, tracheal respiration, zero pressure line and time in 6 seconds Graph *d* shows the effect of injecting 10 mg of plumbagin through femoral vein Graph *e* shows the effect of similar dose after both vagi had been cut Graph *f* shows the same after atropinization

The excised heart of a rabbit perfused with 1 in 800,000 solution of plumbagin showed an initial increase in systole and rate but was soon followed by an irregularity

in rhythm, slowing and decrease in amplitude of contractions With 1 in 400,000 solution a similar effect was shown, but the depression was more marked and the heart's action stopped in about half an hour's time, the auricles stopping before the ventricle (Graphs *h* and *i*) Neither perfusion with Locke's solution, nor injection of 0 25 c c adrenalin were effective in reviving the beat

Frog's heart (*Rana tigrina*) perfused *in situ*, through the inferior vena cava, with R/10 Ringer (NaCl 0 6, KCl 0 024, CaCl 0 0025, $NaHCO_3$ 0 01) showed stimulation with plumbagin and decoction plumbago, in such high dilutions as 1 in 10 million of the former, inasmuch as the heart, which ought to have stopped beating in half an hour's time on this modified Ringer, continued beating for more than an hour The maximum stimulation was, however, produced with 1 in a million strength of plumbagin, when the tone, the rate, the amplitude of contraction and

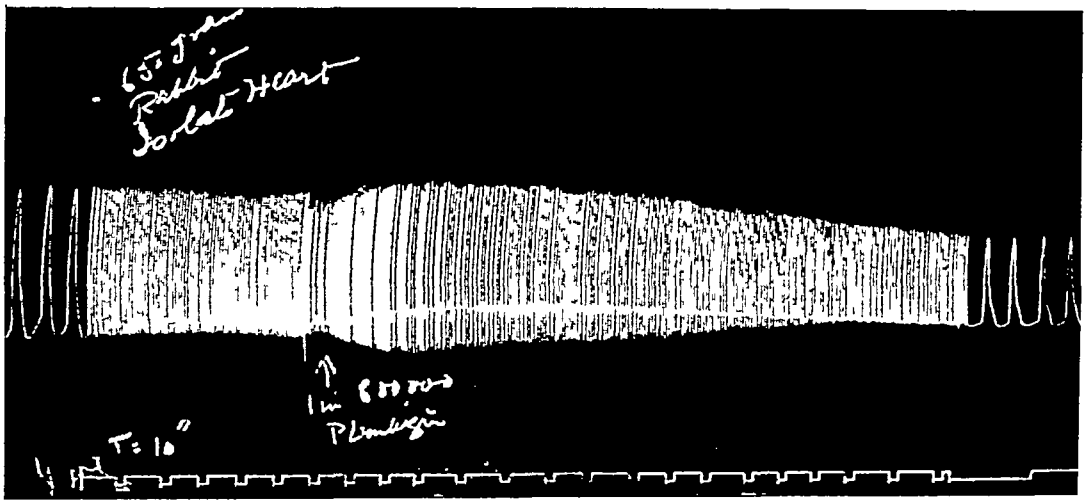


g

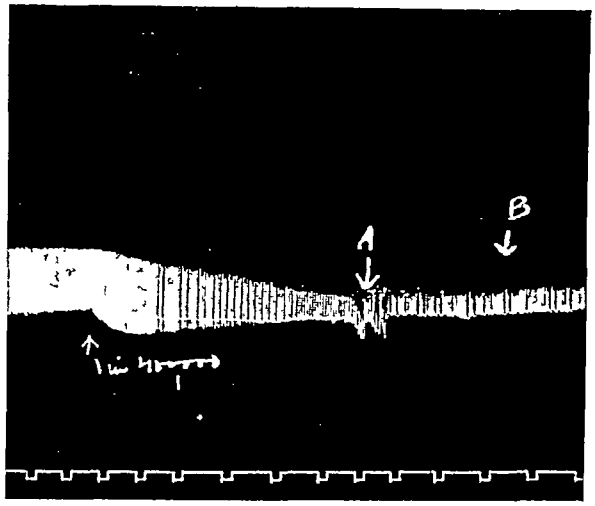
Graph *g* —Frog—perfusion of hind legs, through abdominal aorta (Loewen Trendelenburg's method) Drops recorded with two tambours

the output of the heart markedly increased The stimulant effect came on gradually and lasted a considerable time after the drug was taken off and the heart perfused with R/10 Ringer alone The effect was thus not ' easy-come, easy-go ', but a more lasting one—what Burridge (1920) has described as hysteresis involving changes of aggregation in the colloids concerned in excitation The heart-rate later on became slow and occasionally irregular With higher strength, 1 in 100,000 of plumbagin, there is an initial stimulation followed by gradual depression and stoppage, midway between systole and diastole At this stage the heart-beat could not be revived with full Ringer or adrenalin, but the muscle was still responsive, though feebly, to mechanical stimuli and 5 per cent KCl solution Thus there was failure of excitation, as well as impairment of contractility

Respiratory system —Plumbagin in $\frac{1}{4}$–$2\frac{1}{2}$ mg doses in dogs showed slight broncho-constriction Larger doses of 7–10 mg produced irregular and slow respiration, leading to complete failure—the heart continuing to beat



h



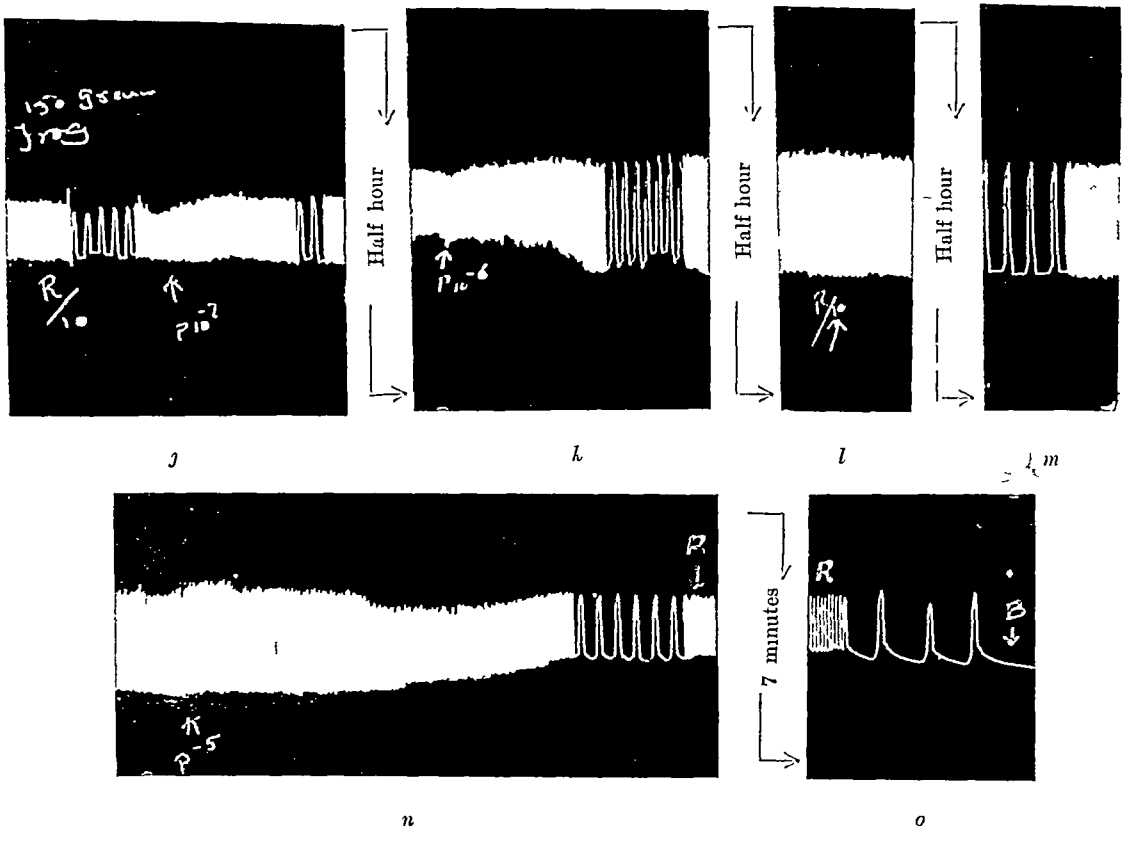
i

Graphs *h* and *i* —650 grammes Rabbit—isolated heart, perfused, with Locke's solution (Lagendarf's method), upstroke=systole Time 10 seconds Graph *h* shows the effect of perfusion with 1 in 800,000 solution of plumbagin Graph *i* with 1 in 400,000 At A changed to Locke, at B 0 25 c c of adrenalin injected Ten minutes later heart-beat completely stopped Heart muscle was still responsive to mechanical stimuli though feebly

Urinary system —Perfusion of an excised sheep's kidney with 1 in 100,000 of plumbagin, showed dilatation of blood-vessels and increase in the ureteral outflow

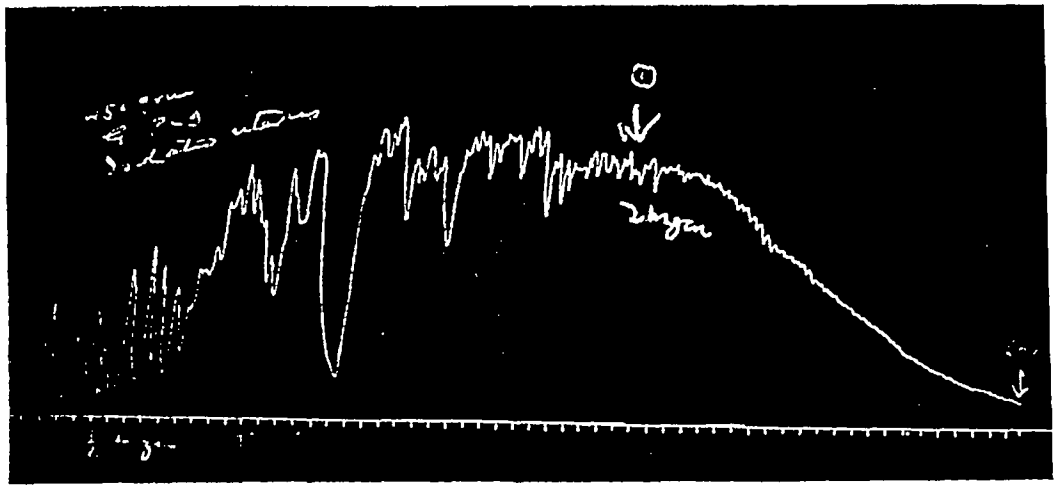
Outflow through the ureter of a dog (under morphine urethane anæsthesia) also showed slight increase in the number of drops of urine per minute

Genital system —In the excised virgin or parous, but non-pregnant, uterus of a rabbit or guinea-pig (Magnus' method) suspended in 1 in 400,000 to 1 in 100,000

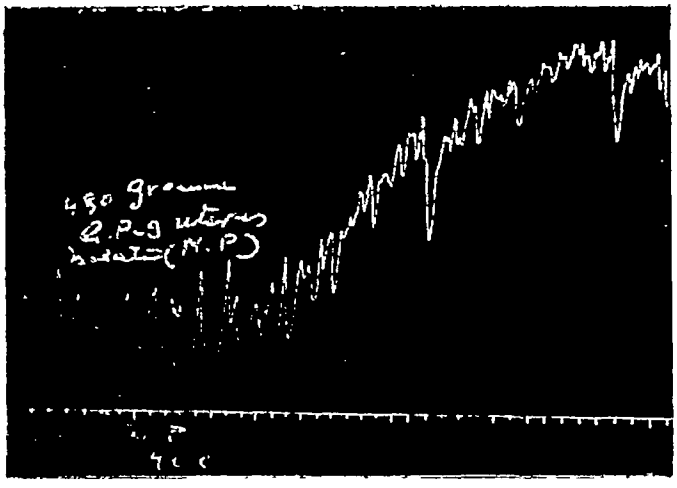


Graphs *j*, *k*, *l*, *m*, *n* and *o* —150 grammes Frog (*Rana tigriana*)—perfused through inferior vena cava at room temperature (33°C) Composition of R/10 0 6 per cent NaCl, 0 03 per cent KCl, 0 0025 per cent $CaCl_2$, 0 01 per cent $NaHCO_3$ Height of reservoir 12 cm —in Graph *j* at P 10^{-7} heart perfused with 1 in 10,000,000 of plumbagin Graph *k* which records the events half an hour later, heart still beating at the initial height at P 10^{-6}, solution changed to 1 in 1,000,000 plumbagin Graphs *l* and *m* show the effect of return to R/10 Graph *n* at P 10^{-5}, heart perfused with 1 in 100,000 of plumbagin Graph *o* at R, changed to Ringer 0 6 per cent NaCl, 0 03 per cent KCl, 0 025 per cent $CaCl_2$, 0 01 per cent $NaHCO_3$ At B 0 25 c c of adrenalin injected—no further automatic contraction was obtained, though the heart muscle still responded feebly to mechanical stimuli and 5 per cent KCl

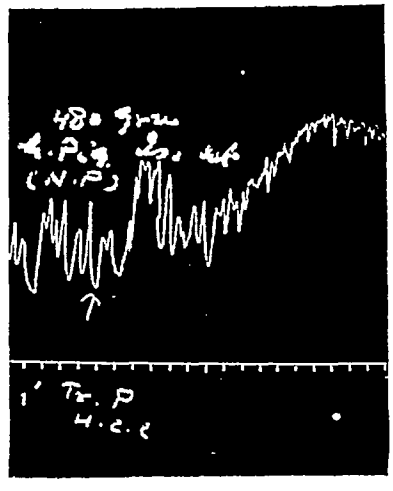
solutions of plumbagin in Locke's fluid the tone of the organ, amplitude and frequency of contractions is increased Exactly similar results were observed with decoction and tincture in 1 in 100 to 1 in 50 dilutions With higher doses of



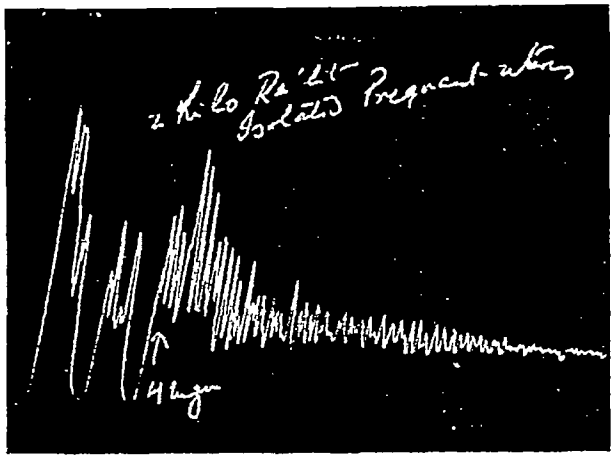
p



q



r



s

Graphs *p*, *q*, *r* and *s* —Guinea-pig—excised, non pregnant parous uterine horn suspended in 200 c c of Locke's solution (Magnus' method), upstroke=contractions Time in one minute Graph *p* shows effect of 1 in 400,000 solution of plumbagin at (1), of 1 in 100,000 plumbagin at (2), adrenalin ½ c c of 1 in 1,000 and later barium chloride 1 c c of 5 per cent solution, were added without any effect Graph *q* shows effect of suspension in 1 in 50 of decoction. Graph *r* shows effect of suspension in 1 in 50 of tincture Graph *s* shows effect of ¼ mg of plumbagin in 200 of Locke's solution on the pregnant excised uterus of rabbit

plumbagin (1 in 50,000) there is complete relaxation of uterine strip, with disappearance of rhythmic movements and setting in of paralysis (Graphs *p*, *q* and *r*) Excised pregnant uterus of guinea-pig or rabbit behaved exactly in the same manner as non-pregnant ones, as regards stimulation with lower and paralysis with higher doses But neither small nor larger doses of plumbagin or decoction given orally, intravenously or subcutaneously to pregnant rabbits or guinea-pigs, succeeded in producing abortion in our series of observations The doses were pushed to the extent of producing mild toxic symptoms

Central nervous system —In frogs, small doses 0 001 mg per gramme weight, showed stimulation , higher doses showed drowsiness and ultimate paralysis No convulsion or tetanic movements were observed Osseous muscles of frogs showed stimulation with smaller and depression with higher doses

Secretions —Injection of 1 mg of plumbagin in the dorsal lymph-sac of a frog, weighing 75 grammes, produced a fair amount of diaphoresis Secretion of urine and bile was also slightly stimulated in dogs, with 1 mg doses of plumbagin administered through the femoral vein

Summary and conclusions

1 That the active principle of *Plumbago zeylanica* is plumbagin and that most of its pharmacological actions are due to the presence of this neutral principle

2 Externally it is a strong irritant and has a powerful germicidal action on bacteria and unicellular organisms

3 The principal action of plumbagin is on the muscular tissue which it stimulates in smaller doses and paralyses in larger ones

4 It stimulates the contraction of the muscular tissue of the heart, intestines and uterus This action is deep-seated

5 It stimulates the secretion of sweat, urine and bile

6 It has a stimulant action on the nervous system

Thus the use of *Plumbago zeylanica* in indigenous medicine, as a rubefacient, vesicant, local ecbolic, diuretic, and sudorific, has a rational basis

We wish to express our thanks to Rai Bahadur Dr B N Vyas, Head of the Department of Pharmocology, for constant guidance

One of us (S L) wishes to express his thanks to the Trustees of Kunwar Inderjit Singh Research Fund for a scholarship which enabled him to take part in this investigation

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SOME APPLICATIONS OF THE SPECTROSCOPE IN MEDICAL RESEARCH

BY

LIEUT-COLONEL T C BOYD, I M S,

AND

N K DE, M SC,

Biochemical and Spectroscopic Research Workers under the Indian Research Fund Association

(*From the Department of Chemistry, Medical College, Calcutta*)

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THE use of the spectrograph has within recent years been clearly demonstrated in connection with analytical problems, associated particularly with metallurgical chemistry The method in selected cases is much more rapid and searching than a detailed chemical analysis and, in addition, lends itself to actual quantitative estimations It is only natural therefore that in biochemical work, where infinitesimal amounts of a substance may prove to be of the greatest importance, the method should also receive its due share of consideration, already, in fact, a certain amount of work has been published on the subject in this respect by Ramage (1930), Ramage and Fox (1931), Chaston-Chapman (1930), McCollum (1929), and others Unfortunately, many laboratories are handicapped in work of this nature owing to the cost of suitable apparatus We have been exceedingly fortunate at having placed at our disposal a high dispersion quartz spectrograph, through the kindness of Sir C V Raman, F R S, Nobel Laureate, and in addition receiving a grant from the Indian Research Fund Association It is not proposed to discuss in this paper the various applications of spectrography to medical problems but to confine ourselves strictly to those problems which have a special bearing on biochemical analysis To make the subject more lucid the paper has been divided into two heads —

(1) Introductory considerations

(2) Qualitative analysis

(1) Introductory considerations.

Under this we may consider what the term spectrographic analysis implies, the type of instruments suitable, methods for the production of spectra and the identification of spectral lines All spectrographic analysis depends on the emission of spectral lines which are specific for the excited element It may happen that the lines produced by different elements overlap or lie in very close approximation, hence the necessity of using a high dispersion instrument, where a variety of elements are liable to be encountered Iron, such a common ingredient in biochemical substances, is particularly troublesome owing to the large number of spectral lines that it exhibits Much spectrographic work can be carried out by using the smaller dispersion instruments, but they are largely applicable to cases where the number and type of the elements sought are limited Again, we have to consider the question of wave-lengths over which it is desirable to examine the spectral lines Many observations can be carried out using an instrument with glass optical parts, but the observations will be limited to roughly between 7,500 and 4,000 Angstrom Units, so, if we wish to extend our observations beyond the violet, an uviol or quartz optical train is essential Bardet in his 'Atlas de arc spectral', has pointed out the following advantages obtained from observations over the shorter wave-lengths, briefly these are —

(*a*) In the ultra-violet region the lines are more dispersed than in the visible, hence their identification is much easier

(*b*) The cyanogen bands practically occupy the region 4,600 to 3,500 Though we have followed Bardet's technique closely, we have not limited ourselves to any particular range of wave-lengths

For general biochemical spectrographic analysis the apparatus that we have found the most suitable is a high dispersion spectrograph with a quartz optical train

General methods employed for exciting spectra

These may be considered briefly under the heads (*a*) Flame spectra, (*b*) Spark spectra, and (*c*) Arc spectra, other methods of excitation being omitted

(*a*) *Flame spectra* —The use of the Bunsen flame itself is extremely limited Ramage has, however, by the use of the oxy-coal gas or oxy-hydrogen flame, been able to detect a considerable number of elements and notes, by using his technique, that the lines are few compared to the arc spectra and so much easier to identify Lundegardh, using a mixture of air and acetylene, mentions about twenty elements that can be detected His method depends on the atomization of a solution of the substance under examination which is passed into the flame Present-day advances in the study of flame spectra make this method of excitation one that cannot be neglected

(*b*) *Spark spectra* —These result when there is a removal of an electron from an atom which has already lost an electron The method is extremely useful where solutions are used but, in addition to the spectrograph, additional apparatus is required in the shape of a powerful coil, or better still, a transformer, a good capacity condenser and a Hemsalech coil to cut out air-lines A very large and important literature exists on this method of excitation

(*c*) *Arc spectra* —These result when an electron is removed from a neutral atom We have already stated that we have followed Bordet's technique fairly closely and have limited our experiments largely to this method of excitation In addition, we may add that the research staff of Messrs Adam Hilgers have pointed out that the arc method of excitation is often more useful than the spark method in detecting very small quantities of a metal

Identification of lines and their persistence

Hartley was the first (working with spark spectra) to observe that the most marked lines emitted by an element were not always the ones that persisted on dilution and, utilizing this observation, he was able to form a quantitative idea of the presence of an element in a compound DeGramont has done a considerable amount of most important work on what he styles the Rais ultimes It is hardly necessary to state that for analytical work it is essential to know the position of these persistent lines and also their sensitivity or dilution at which they disappear Excellent tables are available for this purpose Recently Ryde and Jenkins have produced a powder known as R U powder which contains small quantities of about fifty elements, so worked out that the R U lines of these elements appear on arc excitation We are constantly using this powder and find it most useful in the detection of elements It may be advisable to pause here and ask if spectral analysis can be advocated in attacking biochemical problems more especially now with the advent of and rapid advances made in micro-chemical methods , the answer must certainly be in the affirmative We know little at the moment of the possible importance of minute amounts of an element in an animal or plant tissue , many are probably extraneous, gathered in by accident so to speak from the environment, others may play a most important rôle in the organism and still await elucidation of their action Certainly we cannot neglect the rôle of the elements in catalytic phenomenon and, when all is said and done, we must still admit that the continuation of our being depends largely on enzymes, to conduct a systematic search for elements by micro-methods, would be extraordinarily time-consuming and, it may be added, suitable micro tests do not exist for all elements Admittedly the spectrographic method does not surmount all these problems, as in many cases the method is supersensitive and the possibility of contaminations very difficult to avoid, while in other cases it is under-sensitive and cannot compete with micro-chemical

methods The actual range covered, in which the method is suitable, is large, and the time taken over the analysis by a skilled worker is extremely short We, therefore, consider it a most useful adjunct in certain lines of attack on biochemical problems

(2) Qualitative analysis.

We may now proceed to the application of the method in qualitative analysis, using the arc excitation This in its simplest form consists of applying a portion of the substance in a state of ash, or powder on the positive electrode in a suitable arc As electrodes, we employ H S brand graphite supplied by Messrs Adam Hilger as they carry an analysis of the impurities that they contain In many examinations we use carbon-rods purified in the laboratory, electrodes of silver, copper and iron are used in certain cases, the voltage used is 220 D C and the amperage we vary according to the analysis, usually it is about 3–5, but for the visible region we often run up to 7 or 8 amperes As a working rule we increase both the current and time for the controls over that for the actual sample with the object of showing up impurities in the electrodes themselves

Many methods may be used to prepare the substance in a state of ash, the easiest is simply to ash in a platinum or silica crucible, using a furnace or iron burner If the ordinary Bunsen is used, contamination by copper will result It should also be remembered that platinum is capable of forming an amalgam with many metals at high temperatures The drawback to this method of ashing is of course obvious, where volatile elements like arsenic, antimony, mercury, lead, etc, are sought We have endeavoured to surmount this difficulty by ashing in an enclosed silica tube and collecting all the products of combustion The process is very tedious and we have not been very successful Another method that appears promising, as far as our experience at present shows, is a combination of the chemical and spectrographic method, in other words, chemical group separation and an examination of the group by the spectroscope This would appear to be very useful where only the small dispersion spectroscope is available, in fact we often find that by removing iron chemically, we get excellent results with the small quartz apparatus This method of group separation appears to be finding favour with Italian and Japanese workers

As far as this paper is concerned, we are limiting ourselves to the simple ashing process and embedding the ash in a drilled graphite-rod For purposes of identification of the lines we use a spectrum of R U powder and a spectrum of iron The usual plan adopted is first to take a spectrum of the graphite-rod, to see what impurities are present, we then take three spectra one of iron, one of the unknown, and one of R U powder Some of the actual prints taken from the plates are shown in Plate XXXI Using this simple technique we have examined some human organs The results obtained are noted in Table I In addition, we have

Print taken from an original kidney spectrogram

(*a*) is the iron spectra (*b*) is the spectra of R U powder (*c*) is kidney ash

Print taken from an original puin shak spectrogram

(*a*) is the iron spectra (*b*) is the puin shak ash (*c*) is the R U powder

Print taken from an original musuri dhal spectrogram

(*a*) is the iron spectra (*b*) is the dhal spectra (*c*) is the R U powder (*d*) is the graphite electrode spectra

Part of the above kidney spectrogram enlarged with the important wave-lengths marked

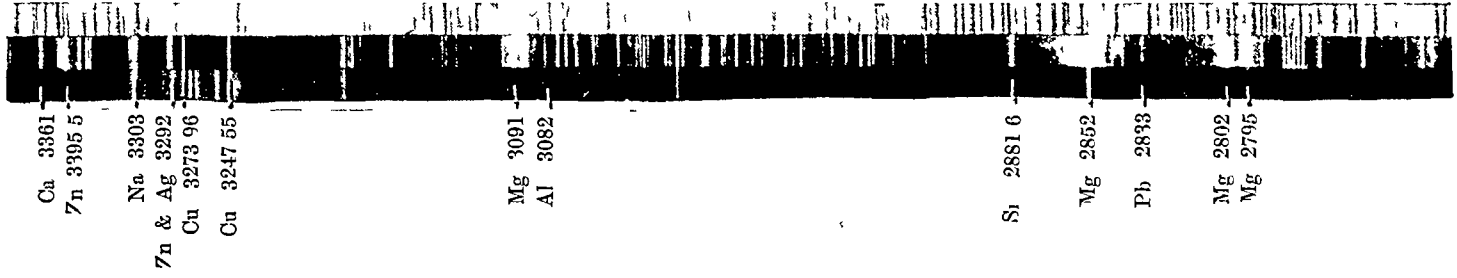
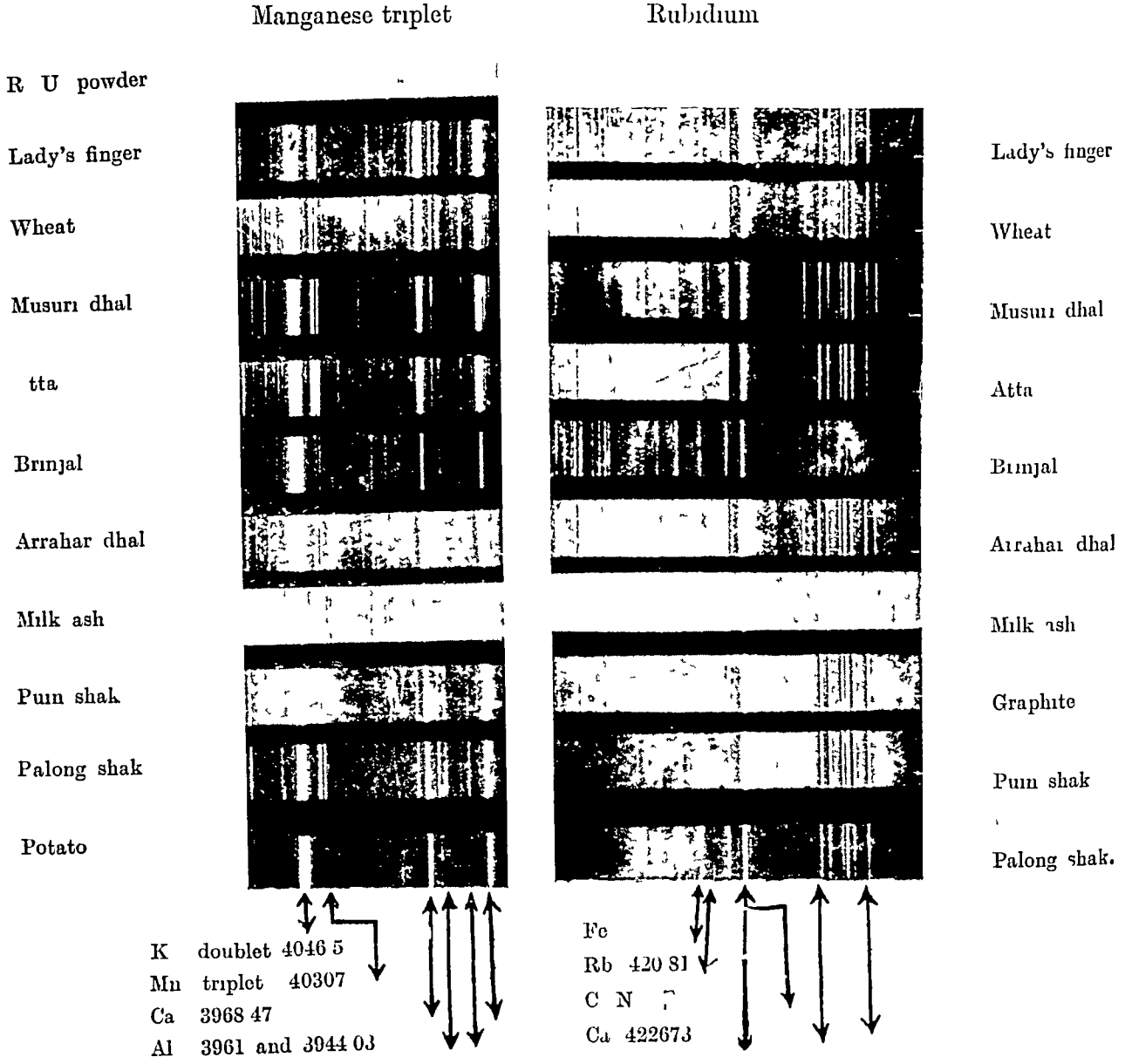


Plate XXXII

This shows two enlargements taken from spectrograms giving the position of the manganese triplet 4034 45, 4033 07 and 4030 76 the enhancement of the lines is clear in certain cases and also the position of the rubidium line 4201 81 This is much more difficult to decipher on the enlargement



The left-hand picture also shows the presence of aluminium doublet 3961 54, 3944 03 This seems to be very common in human organs and food-stuffs

also examined many Indian food-stuffs, the results are noted in Table II In Plate XXXII are shown some of the enlargements obtained from actual plates showing the presence of certain elements found in the substances under examination

TABLE I

	HUMAN ORGANS
Pancreas (Ashed in Silica)	Manganese, triplet 4034 45, 4033 07 and 4030 76 well marked (sensitivity 3*a*, 3*a* and 4*a*) Zinc (McCollum, Rask and Becker, 1928), lines 4810 53 (sensitivity 50*a*), 4680 14 (sensitivity 30*a*) absent Aluminium, 3961 54 (sensitivity 7*a*), 3944 03 (sensitivity 5*a*), 3082 16 (sensitivity 3*b*), lines were present Lead, lines 4057 83 (sensitivity 10*a*), 3639 58 (sensitivity 6*a*), 2614 2 (sensitivity 4), 2833 07 (sensitivity 5*a*) Copper, doublet 3247 55 and 3273 96 (sensitivity 20*a*, and 15*a*) Rubidium, line 4201 (sensitivity 4*a*) Silver line 3382 89 (sensitivity 10*a*) Tin, line 2863 (sensitivity 4*a*) Vanadium, group 1379 to 4408 (sensitivity 2 and 1), appears to be enhanced Cobalt, absent Nickel, absent Strontium, absent
Brain (Ashed in Silica and in Platinum)	Manganese, triplet present but very faint Zinc, lines 4810 and 4722 Aluminium, lines 3961 and 3944 Lead, lines absent Vanadium, lines absent Silver, line 3382 very marked Rubidium, line 4201 Strontium, lines absent Tin, lines absent Cobalt, lines absent Nickel, lines absent Copper, doublet present
Spleen (Ashed in Silica)	Manganese, triplet well marked Zinc (McCollum, Rask and Becker, 1928), lines 4810 and 4722 Aluminium, lines 3961 and 3944 Lead, 3639 and 4051 Copper, doublet present Silver, lines 3382 Rubidium, line 4201 Tin, line 2863 Cobalt, lines absent Nickel, (Martin, 1930), lines absent Vanadium ? Strontium absent
Kidney (Ashed in Platinum)	Manganese, triplet well marked, also lines 2593 and 2798 (sensitivity 1 and 1) Zinc, lines 4810, 4722, 4680, 3345 (sensitivity 50*a*, 50*a*, 30*a*, 30*b*) Aluminium, 3961, 3944, 3082 and 3092 Lead, 2833 Copper, doublet enhanced Vanadium, group enhanced Silver, 3382

TABLE I—*concld*

	HUMAN ORGANS
	Tin, 2839, 2863 (sensitivity 4*a*) Rubidium, 4201 Cobalt, absent Nickel, absent Cæsium, absent Barium, absent Beryllium, absent
Liver, different samples (Ashed in Silica and Platinum)	Manganese, triplet well marked Zinc, lines 4810, 4722, 4680 and 3345 Aluminium, 3961, 3944 and 3082 Lead, lines well marked 4051, 3639, 2833 Copper, doublet present and enhanced Vanadium, group enhanced Silver, 3382 Strontium, lines absent Cobalt, lines absent Nickel, lines absent Tin, line 2863 Rubidium, line 4201 Beryllium, lines absent Cæsium, lines absent
Heart (Ashed in Platinum)	Manganese, line 2593 Aluminium, line 3082 Lead, line 2833 Zinc, line 3345 Copper, doublet Silver, line 3382 Tin, lines absent
Muscle (Ashed in Silica)	Manganese, 2593 Aluminium, 3082 Lead, 2833 Zinc, 3345 Copper, doublet Tin, absent Silver, line 3382

TABLE II

	INDIAN VEGETABLES
Bengal Rice (Ashed in Platinum)	Manganese, triplet, 4034 45, 4033 07 and 4030 present Zinc, lines 4810, 4722 4680, 3345 Aluminium, lines 3691, 3944, 3082 Lead, lines 4057, 3639, 2833 Copper, doublet present Rubidium, line 4215 Silver, lines absent Tin, lines absent Vanadium, group 4379 to 4408 enhanced Cobalt, lines absent Nickel, lines absent Strontium, line 4078 Beryllium, line 3321

TABLE II—*contd*

INDIAN VEGETABLES

Rangoon Rice (Ashed in Platinum)	Manganese, triplet present Zinc, lines absent Aluminium, lines (doublet) present Lead, lines 4057, 3639, 2833 Copper, doublet present Rubidium, line 4215 Silver, lines absent Tin, lines absent Vanadium, group enhanced Cobalt, lines absent Nickel, lines absent Strontium, line 4078 Beryllium, line 3321
Wheat (Ashed in Platinum)	Manganese, triplet enhanced *Zinc, lines 1810, 4722, 4680 present* Aluminium, doublet present Lead, lines 4057, 3639 Copper, doublet present Rubidium, line 4215 Silver, line 3382 Tin, lines absent Vanadium, group enhanced Cobalt and Nickel absent Strontium, line 4078 very marked Beryllium, line 3321
White and Brown Atta (Ashed in Platinum)	Manganese, triplet very enhanced Zinc, triplet present Aluminium, doublet present Lead, lines 4057, 3639, 2833 Copper, doublet present Rubidium, line 4215 Silver, line 3382 enhanced Tin, lines absent Vanadium, group present Cobalt and Nickel absent Strontium, line 4078 enhanced Beryllium, line 3321
Musuri Dhal (Ashed in Platinum)	Manganese, triplet present Zinc, triplet present Aluminium, doublet well marked Lead, lines absent Copper, doublet present Rubidium, line 4214 Silver, line 3382 enhanced Tin, lines absent Vanadium, group enhanced Cobalt, Nickel and Strontium absent Beryllium, line 3321
Mug Dhal (Ashed in Platinum)	Manganese, triplet enhanced Zinc, lines 4810, 3345 Aluminium, doublet present Lead, lines 4057, 3639 Copper, doublet present

TABLE II—*contd*

		INDIAN VEGETABLES
		Rubidium, line 4201 Silver, line 3382 well-marked Tin, lines absent Vanadium, group enhanced Cobalt, Nickel and Strontium absent Beryllium, line 3321.
Arrhar Dhal (Ashed in Platinum)		Manganese, triplet present Zinc, line 4810 Aluminium, doublet present Lead, lines absent Copper, doublet present Rubidium, line 4201 Silver, line 3382 Tin, lines 2839, 2863 Vanadium, lines enhanced Cobalt, Nickel and Strontium absent Beryllium, line absent
Beuli Dhal (Ashed in Platinum)	..	Manganese, triplet present Zinc, lines 4810, 4722, 3345 Aluminium, doublet enhanced Lead, line 4057 Copper, doublet present Rubidium, line 4201 enhanced Silver, line 3382 well marked Tin, lines absent Cobalt and Nickel, absent Strontium, line 4078. Beryllium, absent Vanadium, group enhanced
Potato (Ashed in Platinum)	.	Manganese, triplet present Zinc, lines 4810, 4722, 4680, 3345 Aluminium, doublet present Lead, lines absent Copper, doublet present Rubidium, line 4201, 4215. Silver, line 3382 Tin, lines absent Cobalt, Nickel and Strontium, absent. Vanadium, group present. Beryllium, absent.
Brinjal (Ashed in Platinum)		Manganese, triplet very faint Zinc, lines absent Aluminium, doublet very faint Lead, absent Copper, doublet present. Rubidium, line 4215 Silver, lines absent Vanadium, group absent Tin, present 2863 Cobalt and Nickel, absent Strontium, present 4078 Beryllium, absent

TABLE II—*concld*

	INDIAN VEGETABLES
Lady's Finger (Ashed in Platinum)	Manganese, triplet present Zinc, absent Aluminium, doublet very faint Lead, absent Copper, doublet present Rubidium, line 4215 Silver, line 3382 Vanadium, group faint Tin, lines absent Beryllium, line absent Strontium, line 1078
Puin Shak (Ashed in Platinum)	Manganese, triplet present Zinc, lines absent Aluminium, doublet present Lead, lines absent Copper, doublet present Rubidium, line absent Silver, line 3382 enhanced Tin, lines absent Vanadium, group present Cobalt and Nickel, absent Strontium, line 4078 present Beryllium, line absent
Palong Shak (Ashed in Platinum)	Manganese, triplet enhanced Zinc, lines absent Aluminium, doublet present *Lead, lines absent* Copper, doublet present Rubidium, line absent Silver, line 3382 enhanced Tin, lines absent Vanadium, lines absent Cobalt and Nickel, absent Strontium, line 4078 present Beryllium, line absent.

Note —It will be seen that under the head Pancreas, sensitivity is mentioned for most of the main lines of the elements These sensitivity notations have been taken from Adam Hilgers' booklet 'Sensitive arc lines of fifty elements and the use of R U powder in spectroscopic analysis' The suffix (*a*) means most sensitive, (*b*) the next, and so on

SUMMARY OF RESULTS

These may be considered under two heads (1) Human organs and, (2) Indian food-stuffs

(1) *Human organs*

Manganese This element is very widespread in all organs but is particularly well marked in the pancreas, kidney and liver The lines are very faint in the case of brain ash

Zinc This element is also widespread and very marked in the kidney and liver

Aluminium (Dee Tourtellotte and Rask, 1931) This is also widespread and well marked in the kidney and liver

Lead This is well marked in the liver and is also present in all the other organs examined except in the brain

Iron Present throughout

Copper Present throughout

Rubidium This element appears to be common

Silver also common and particularly well marked in the brain

Strontium (Chaston-Chapman, 1930), *Cobalt, Nickel* (Martin, 1930) and *Barium* were found to be absent throughout

Tin (Bertrand, 1931) This was found in the pancreas, kidney, liver, spleen but absent in muscle, brain and heart

Vanadium The lines of this element were found to be enhanced in the kidney and liver but appeared to be absent in the spleen and brain

(2) *Indian food-stuffs*

Manganese This element was found to be present throughout but the lines were found to be enhanced in wheat, atta, mug dhal and palong shak

Zinc This was found to be present in Bengal rice, wheat, atta, musuri dhal, arrhar dhal, beuli dhal, potato, but was not found in Rangoon rice, brinjal, lady's finger, puin shak, and palong shak

Aluminium This element was present throughout but was well marked in Bengal rice, musuri dhal, beuli dhal, while it was very ill defined in brinjal and lady's finger

Lead Lines were found in Bengal rice, Rangoon rice, wheat atta mug dhal and beuli dhal only.

Iron and *Copper* were present in all the samples examined

Rubidium This was found in all samples except puin shak

Silver This was found to be present in all the materials examined except in rice and brinjal It was well marked in atta, musuri dhal, mug dhal, beuli dhal, and puin and palong shaks

Tin This was only found in arrhar dhal and brinjal

Cobalt, Nickel and *Barium* were absent throughout

Vanadium This was well marked in rice, wheat and dhal It was absent in brinjal and palong shak

Strontium (Fox and Ramage, 1930) This was found to be well marked in wheat and atta and absent in musuri dhal, arrhar dhal and potato

Discussion

We may now compare our results with those of previous workers in this field *Aluminium* The presence of this element in plants and animal tissues appeared to be a disputed point, for sometime McCollum, Rask and Becker (1928) considered from their work that this metal was not a constituent, while Kahlenbergh and Closs (1929) found it to be universally present Our results agree with the latter workers *Manganese* For this element our results agree with Bertrand and Rosenblatt (McCarrison, 1927) and also it may be noted that the lines were enhanced in the liver, kidney and pancreas in the case of the organs and in wheat, atta, mug dhal and palong shak in the food-stuff series It is particularly interesting to draw attention to the work of McCarrison (1927) on the effect of this element in the food-stuffs and its bearing on the growth of animals, our results would appear to endorse his ideas that the excess of this element might be an important factor in the physical build of different people using different types of food-stuffs *Strontium* This element is common in vegetable substances but we have been unable to detect it in the human organs (Ramage and Fox, 1930)

The results of our spectroscopic analysis, we think, shows that the question of the presence and possible function of minute amounts of elements present in organs and plants still requires a large amount of research in feeding experiments, the rôle of these elements is still very obscure though possibly light is apparent in the case of copper and manganese, but we yet know little whether some of these elements present are purely accidentals or whether they play a specific rôle, possibly inter-changeable, in their function in different animals and plants The presence of some assuredly may be ascribed to contaminations from food-stuffs, such as in the case of lead, but we are not so certain when we consider the question of vanadium or even silver We may yet have to prove which in fact are essentials and which accidentals In that work spectrographic analysis should prove most useful and we can only at the moment summarize the results obtained by us

Our best thanks are due to Professor Sir C V Raman for his interest and advice in the work We also thank Dr B Chakraborty, present Chemical Examiner to Government, Bengal, for his kindly supplying us with necessary materials

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Ind Jour Med Res, XX, 3, *January*, 1933

A CASE OF GYNANDROMORPHISM IN *SIMULIUM*

BY

I M PURI, M SC (Punjab) PH D (Cantab), F E S,

Officer In-Charge, Inquiry on the Indian Simulidæ, Culicoides and other Blood-Sucking Midges

(From the Central Research Institute, Kasauli)

[Received for publication, July 27, 1932]

ABNORMALITIES are by no means common among adult Simulidæ Out of hundreds of specimens bred out of pupæ only a single individual, hatched out of an isolated normal pupa belonging to *S grisescens* var *palmatum* Puri, was peculiarly modified, showing secondary sexual characters of both sexes This gynandromorph specimen is interesting in having the head of a female, the thorax half male and half female while its abdomen is typically male

Gynandromorphism or the presence of characters of both the sexes in the same individual has often been observed in a number of families of insects A large number of such abnormal individuals have been recorded among Lepidoptera, and Keilin and Nuttall (1919) have described different types of gynandromorphism in *Pediculus humanus* According to them, Cockayne (1916) has classified the various types of gynandromorphs into three groups

I Genetic hermaphrodites, in which the primary sex glands of both sexes are present

II Primary somatic hermaphrodites which possess gonad or gonads of one sex only, but parts of the secondary sexual apparatus, internal or external of both sexes are present

III Secondary somatic hermaphrodites, which possess gonad or gonads of one sex and the secondary sexual apparatus of that sex but have secondary sexual characters of both sexes

Majority of the abnormal individuals described before conform to group II of Cockayne and are of the nature of 'halved gynandromorphs' Although the gonads of the gynandromorph specimen of *Simulium grisescens* var *palmatum* were not dissected out, it seems obvious from the secondary sexual characters described below in detail that this individual belongs to the third category of Cockayne

SPECIMEN No 7827

Head resembles that of female *grisescens* in every character The antennæ are brownish yellow with the distal three to four segments nearly brownish black, the whole with a pale pubescence

Thorax —(Plate XXXIII, fig 1); *Right half* (*A*) of mesonotum velvet black resembling that of a male *grisescens* in every way (covered with fine copper-coloured pubescence, in the fore-corner a shimmering silvery spot, only half of it being seen at a time, a broad ash grey border externo-laterally and also posteriorly in certain lights)

Left half (*B*) of mesonotum is like that of a typical female in colour as well as in ornamentation The golden pubescence covering it is comparatively coarser than that on the right half The black stripes on a dull ash grey surface, characteristic of the species are very clear and well defined

Abdomen (including the genital armature) is identical with that of a typical male *grisescens*

Legs —The right hind leg is unfortunately broken off in this specimen and the colour of the legs in the two sexes is practically the same in this species but an examination of the claws shows that those on the right legs (Plate XXXIII, fig 2) are like the ones found in the males (with a small basal hood), while those on the left legs are simple, resembling the female claws of *grisescens* (Plate XXXIII, fig 3)

Wings as in *grisescens* —The basal portion of the radius proximal to the fork on the right wing (Fig 4) is bare, as found in the male, while on the left (Fig 5) it is hairy resembling the condition found in the female of this species

PUPA.

The pupa (respiratory filaments and cocoon) resembles that of a typical *S grisescens* var *palmatum* in every character

This specimen was bred out of an isolated pupa collected, along with a very large number of others belonging to this variety, to *S striatum* Brunetti and to *pattoni* S W, from the rushing waters of the Cauvery River near Frazerpet, Coorg, (9-1-31)

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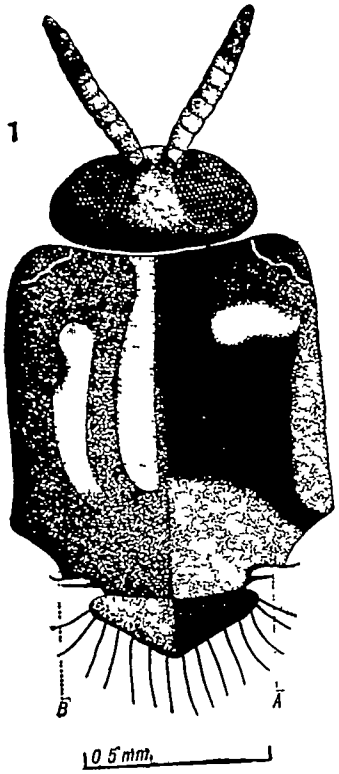
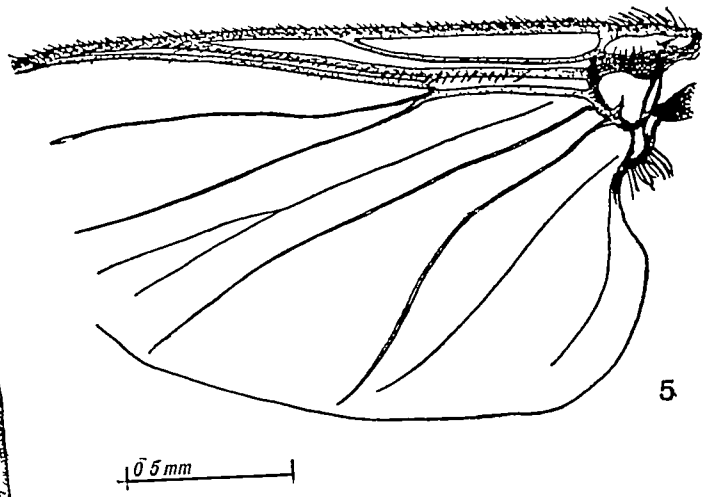
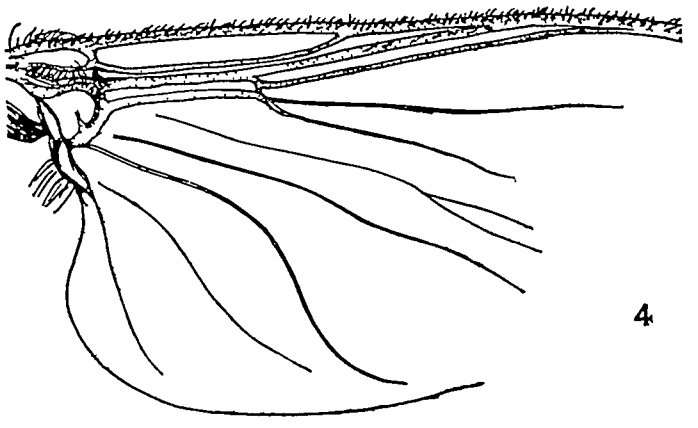
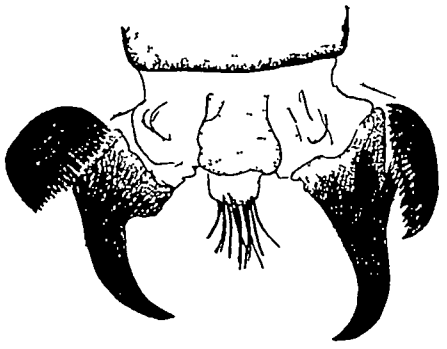
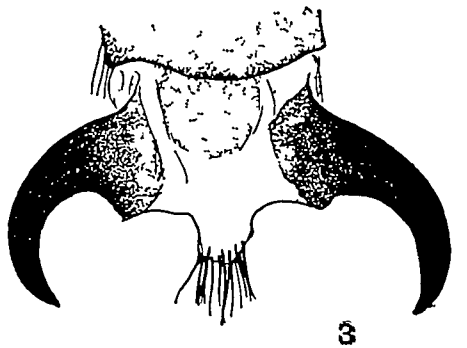
Plate XXXIII











I M Puri *del*

Gynandromorph specimen of S grisescens var palmatum Puri

Fig 1—Dorsal view of head and thorax (meso thoracic pattern as seen from in front)
Fig 2—Tip of right middle leg showing the claws Fig 3—Tip of left middle leg showing the claws
Fig 4—Part of right wing Fig 5—Part of left wing

Ind Jour Med Res, XX, 3, *January*, 1933

STUDIES ON INDIAN SIMULIIDÆ

Part VI

DESCRIPTIONS OF MALES, FEMALES AND PUPÆ OF TWO NEW SPECIES FROM PALNI HILLS AND OF MALE AND PUPA OF *S TENUITARSUS* SP N FROM BENGAL TERAI

BY

I M PURI, M SC (Punjab), PH D (Cantab), F E S,

Officer In-charge, Inquiry on the Indian Simuliidæ, Culicoides and other Blood-Sucking Midges

(*From the Central Research Institute, Kasauli*)

[Received for publication, September 5, 1932]

THE three species described in this paper belong to the sub-genus *Simulium* with somewhat flattened front tarsi and in the two species of which the females are known, the claws of female without any basal tooth and the tip of abdomen shining They are comparatively large in size and are characterized by having simple claws in the females and six respiratory filaments and a large somewhat boot-shaped cocoon in the pupal stage In all three of them the fore tarsi are only very slightly flattened, which distinguishes them from all other species belonging to the above sub-genus so far described in Parts I–V of this study They resemble the species of the *striatum* group dealt with in Part V, in that the outer surface of the fore tibiæ has only an inconspicuous greyish spot instead of the bright silvery spot present in the other species

Simulium (Simulium) gravelyi SP N

FEMALE

Head dark slate, with fine golden pubescence and scattered long black hairs on the occiput, the latter also present in a double row along the lateral borders of the face and the frons Frons dark slate, appearing greyish in certain lights, somewhat shining, comparatively small, narrower than the face, nearly parallel-sided,

very slightly narrowed in the region of the antennæ Face broad, dusted with ash grey The base of the antenna is brownish yellow up to about the middle of the third basal segment, while the rest is nearly black with very fine pale pubescence The colour of the basal portion varies in the different paratype specimens from dirty yellow to nearly reddish Palpi black

Thorax—Mesonotum is covered with a fine golden pubescence (partly rubbed off in the type specimen) When viewed from in front the mesonotum appears grey with three faint brownish black stripes—a very narrow median and two broader sub-median, forming an indistinct lyre-shaped pattern The sub-median stripes are continued along the anterior border and are connected to a still more indistinct dark stripe along the lateral border All these stripes and the grey colour disappear as the specimen is turned forwards and the whole of the mesonotum becomes greenish black, very slightly shining When viewed from behind the posterior one-fourth or so (mostly the prescutellar area) and the posterior ends of the median and sub-median stripes appear ash grey Scutellum brownish black, covered with golden pubescence and having long black marginal hairs Pleuræ slate grey, membranous area bare

Abdomen with the first five segments brownish black (black in most of the paratype specimens), fringe of long hairs on the basal scale golden, dorsum of segment 2 with the usual ash grey reflections, rounded chitinized tergites on segments 3–5 comparatively larger than usual in the sub-genus *Simulium* Tergites of segments 6–8 large, greyish black (black in a number of paratype specimens) somewhat shining, sparsely covered with fine, pale hairs, a few present also on tergites of segments 4 and 5 *Terminalia* (Plate XXXIV, fig 1) Short macrosetæ on the ventral surface of segment 7 scattered on the posterior half, a few forming a group on each side of the median line comparatively stouter than the others Sternite of segment 8 narrow, somewhat ribbon-like, the middle third with the anterior border nearly straight, bearing a few (8–12) macrosetæ on its lateral portions Anterior gonopophyses are of moderate size uniformly covered with minute microsetæ arising in irregular rows and with 6–8 very short slender macrosetæ near their anterior border Their inner borders are somewhat thickened and slightly diverging from each other posteriorly Paraprocts and cerci of moderate size

Legs—Fore coxæ yellow, posterior ones black, all trochanters and femora golden yellow, the latter slightly brownish at the tip Fore tibiæ brownish yellow, gradually becoming dark brown on the distal half to one-third with the distal fourth black, the outer surface with inconspicuous pale grey dusting, tarsi black, very little flattened, first segment about six-and-a-half times as long as its greatest width near its distal end and about as long as the rest of the tarsi together Segments 1 and 3 with a pair of long black hairs subapically on their posterior surface Middle and hind tibiæ golden yellow with distal one-sixth or so nearly black and

a slight whitish sheen on the posterior surface basally, the basal half* of the first and the base of the second tarsal segments of the middle leg golden yellow, while nearly the basal two-thirds of the first and the base of the second tarsal segments of hind leg pale yellow, the rest of tarsi black Pedisulcus well marked, calcipala of moderate size extending up to the former All claws simple The yellow portions of the legs with fine golden pubescence and with some long black hairs

Wings hyaline, radius bare up to the fork, radial sector simple Wing length about 3 25 mm Halteres lemon yellow

The ventral surface of the *buccal cavity* at its posterior end bears a large cluster of minute tubercles The *furca* is comparatively small in size

MALE

Head black, with a fringe of long black hairs on the occiput Face whitish grey, sparsely covered with black hairs Antennæ black with two basal segments brownish yellow (yellowish black in many paratype specimens), the whole with a very fine pale pubescence Palpi black

Thorax—Mesonotum velvet black, covered uniformly and fairly densely with a somewhat coarse golden pubescence† Anteriorly are a pair of large shimmering silvery spots, broadly separated in the middle The whole of these spots reflects light simultaneously (and not only half) In certain lights the mesonotum shows a narrow silvery border laterally and an inconspicuous one posteriorly (better seen in some paratype specimens), the lateral border continuous with the anterior spots Scutellum velvet black, covered with golden hairs and a fringe of long black hairs Membranous area of pleuræ bare

Abdomen velvet black with sparse fine golden hairs on the dorsum, and on segments 3 and 4 a small cluster of long black hairs arising ventro-laterally Long hairs on the basal scale black Silvery spots as usual on segments 2 and 5–7 *Genital armature* (Plate XXXIV, fig 2) Coxites are about as broad as they are long Styles are comparatively long, their length being about $3\frac{1}{2}$ times their greatest width near the base Beyond their basal one-third they become slightly narrow and are curved inwards distally From their dorso-internal surface near the base, each of the styles sends upwards and inwards a fairly large triangular process, which bears strong teeth distally and along its anterior border Each style bears a single short sub-terminal spine on its inner edge The inter-coxal piece (Plate XXXIV, fig 3) has a moderately broad base (*b*), about two-thirds as wide as the distance between the ends of the two inner arms or the apodemes (*a*) and from which a somewhat conical process is produced downwards This process

* About $\frac{3}{4}$rds in majority of paratype specimens

† Partly rubbed off in the type specimen

has a rounded end and its anterior and lateral surface is covered with fine setæ, while the posterior which is somewhat flattened is practically bare, having minute setæ only near the distal end The chitinous plate lying above and a little behind the inter-coxal piece is elongated and narrow The cluster of spines on each side of the genital opening is comparatively large

Legs —Fore coxæ pale yellow, posterior ones black, trochanters yellowish at the base, dark grey distally, femora greyish yellow, greyish black near the distal end Fore tibiæ brownish black, black on the distal third, the outer surface with an inconspicuous pale grey spot, tarsi black, very slightly flattened, the first segment being a little over seven times as long as its greatest width near its distal end Middle tibiæ are yellowish brown, brownish yellow on the basal one-third with a whitish sheen on the posterior surface and nearly black on the distal third, basal half of the first tarsal segment yellowish black the rest of tarsi black Hind tibiæ practically black* with a yellowish base, basal half of the first tarsal segment greyish yellow, the rest of hind tarsi black The hind basitarsus (Plate XXXIV, fig 4) is moderately expanded Its length is about 0 75 of that of the hind tibiæ and its greatest breadth is about 0 7 of that of the latter and about a quarter its own length The legs bear coarse bright golden pubescence, best seen on pale portions

Wings as in female Wing length about 3 0 mm.

PUPA

Size about 4 0×1 4 mm

The integument of the head and thorax brown, densely covered with minute disc-like tubercles, except on the region of the metanotum where the tubercles are smaller and sparse Head and thoracic trichomes as in *himalayense*—only 4 pairs of trichomes on the thorax dorsally These are fairly long and simple Dorsal and ventral hooks on the abdomen as in *himalayense*, a pair of well-developed strongly chitinized hooks present on the ventral surface of segment 4 Dorsally along the anterior border of segments 8 and 9 only, is an irregular row of poorly developed cuticular spines, those on segment 9 being better developed than on segment 8

Respiratory filaments (Plate XXXIV, fig 5) are grey in colour, 6 on each side, arranged in three almost sessile pairs The filaments decrease in thickness but increase in length from above downwards The uppermost filament is directed upwards and inwards from its origin and is even less than half the length of the

* In some paratype specimens the hind tibiæ and the first tarsal segment are not so dark The tibiæ are yellowish black on the basal two thirds and black distally, and the basal two thirds of the first tarsal segment is yellow, the rest black

lowermost one which is the longest, nearly half the length of the pupa and is directed practically straight out The surface of the filaments is raised into tubercles of two sizes—the large ones are placed on ridges forming a reticular pattern and the smaller ones are scattered on the interspaces

Cocoon (Plate XXXIV, fig 6) is dirty yellow in colour, closely woven, without any windows and interspaces in the mesh It is boot-shaped with the large irregular opening directed upwards Its lateral and ventro-lateral border is produced forwards as a large flap-like projection on each side and has a deep notch mid-dorsally The length of the cocoon from its posterior end to the anterior border of the opening is about 4 8 mm , the greatest width about 1 6 mm and the extension beyond the base or the attached portion of the cocoon, is about one-third the total length

Described from 31 females and 20 males, all in good condition and nearly all bred out of isolated pupæ collected from the stream above Silver Cascades (24-1-31) and from another large stream below Fairy Falls (25-1-31), both in Kodaikanal (6,000 to 7,000 ft), Palni Hills, South India

Types and paratype in my own collection

I have great pleasure in naming this species *S gravelyi* after Dr F H Gravely, Madras, who first collected large numbers of larvæ and pupæ of this species from Silver Cascades (6,000 ft), Law's Ghat, Kodaikanal, Palni Hills, 17-5-1929 In the stream above Silver Cascades (6,000 ft) I found (25-1-31) the rock in some places covered with innumerable larvæ and pupæ all belonging to this species, the pupæ forming a complete covering, often attached one above the other, two to three deep

Simulium (Simulium) palniense SP N

This species closely resembles *S gravelyi* but the males, females and pupæ show certain distinctive and constant characters on which they can be easily identified from those of the latter species

FEMALE

Head is like that in *gravelyi* but comparatively darker The occiput bears only black hairs which are present on most of the frons also The two basal antennal segments are blackish red, but their colour varies from yellowish black to reddish black in the different paratype specimens, the rest of the antennæ being always black, with very fine pale pubescence

Thorax too is comparatively darker than in *gravelyi* Mesonotum covered with a fine golden pubescence (partly rubbed off in the type specimen) When viewed from in front only the anterior one-third of the mesonotum appears grey (lateral portions paler than the median third) with three very faint dark lines as

in the other species Scutellum nearly black, with some golden pubescence and a number of short and long black hairs along the margin Pleuræ slate grey, membranous area bare

Abdomen (along with terminalia) is as in *gravelyi*, except that the macrosetæ on the ventral surface of segment 7 are practically of the same size, those on either side of the middle line not being stouter than the others, the anterior border of the middle third of the narrow sternite of segment 8 is usually broadly curved, the macrosetæ on this sternite are more numerous, 12–18 in number (Plate XXXV, fig 7) and the macrosetæ on the anterior gonopophyses are comparatively longer than in *gravelyi*

Legs—Fore coxæ greyish yellow, posterior ones black, all trochanters and femora nearly black with somewhat yellowish bases Fore tibiæ yellowish dark grey, gradually becoming black on the distal one-third, the outer surface with inconspicuous pale grey dusting, tarsi black, slightly flattened, the first segment about 7 times as long as its greatest width near its distal end Middle and hind tibia greyish yellow basally, gradually becoming darker distally, black on the distal half with a whitish sheen on the posterior surface basally Basal halves of the first tarsal segments of the middle and hind legs and the base of the second segment of the hind leg yellow, the rest of tarsi black Fine golden pubescence mostly on the pale portions of the legs All claws simple

Wing as in *gravelyi* Wing length 3 14 mm

MALE

Head as in *gravelyi* The two basal segments of the antennæ are brownish black, the rest of the antenna black with fine pale pubescence In a number of paratype specimens the antennæ are all black

Thorax—Mesonotum velvet black, uniformly but sparsely covered with a fine somewhat coppery pubescence (partly rubbed off in the type specimen) Anteriorly are a pair of large shimmering silvery spots broadly separated in the middle Practically the whole of these spots reflects light simultaneously In some paratype specimens the anterior border between the spots and the region just posterior to them is also slightly silvery grey In certain lights the mesonotum shows a silvery lateral and posterior border as well Pleuræ with ash grey reflections

Abdomen is like that of *gravelyi* except that the base of the inter-coxal piece (Plate XXXV, fig 8) is comparatively narrower being only about one-third as wide as the distance between the ends of the arm-like apodemes From the base a narrow keel-like process is produced downwards and forwards, having a flattened postero-ventral surface and rounded finger-like distal end On the proximal two-thirds the lateral edges of the flattened posterior surface are broken up by a row

of poorly developed teeth (Plate XXXV, fig 9), while the anterior surface is covered with backwardly directed setæ which are also present along part of the edge of the posterior surface The distal end of the process is smooth The chitinous plate lying above and a little behind the inter-coxal piece is somewhat widened posteriorly and the cluster of spines on each side of the genital opening is comparatively small

Legs are black, except for a large inconspicuous pale grey spot on the outer surface of fore tibiæ, the bases (practically the joints only) of the middle and hind tibiæ which are whitish, and the yellowish black basal one-third of the first tarsal segment of the hind leg The fore tarsi are very little flattened, the first segment being a little longer than eight times its greatest width near the distal end The hind basitarsus is comparatively more expanded than in *gravelyi*, its length being about 0 76 of that of the hind tibiæ and its greatest breadth about its middle about 0 85 of that of the latter and about 0 28 of its own length (Plate XXXV, fig 10) Fine golden pubescence is present only on the pale portions of the legs

Wings as in female

PUPA

The pupa is of the same type as that of *S gravelyi* except that the thoracic trichomes are comparatively much shorter than in the latter species and that rows of cuticular spines along the anterior borders of abdominal segments 8 and 9 are much better developed and a row, broken in the middle, is also present on segment 7 The *respiratory filaments* are white, about half as long as the pupa, 6 on each side, arranged in three pairs, only the middle one of which appears to have a short stalk All the filaments are more or less of the same thickness and practically of the same length The uppermost filament is directed forwards and a little upwards while the lowermost runs forwards and a little downwards, the rest of them spreading more or less uniformly in between these two The wall of the filaments as in *gravelyi*

The cocoon resembles that of *gravelyi*

Described from 32 males and 27 females all in good condition and bred out of pupæ (only a few from isolated ones) collected together with those of *S gurneyæ* S W, *grisescens* Brunetti and *gravelyi*, from a large stream below Fairy Falls (about 7,000 ft), Kodaikanal, Palni Hills, South India, 25-1-31

Types and paratype in my own collection

Simulium (Simulium) tenuitarsus SP N.

MALE

Head black, with a fringe of short black hair on the occiput. Face whitish grey with scattered black hairs Antennæ dark brown, with the basal three

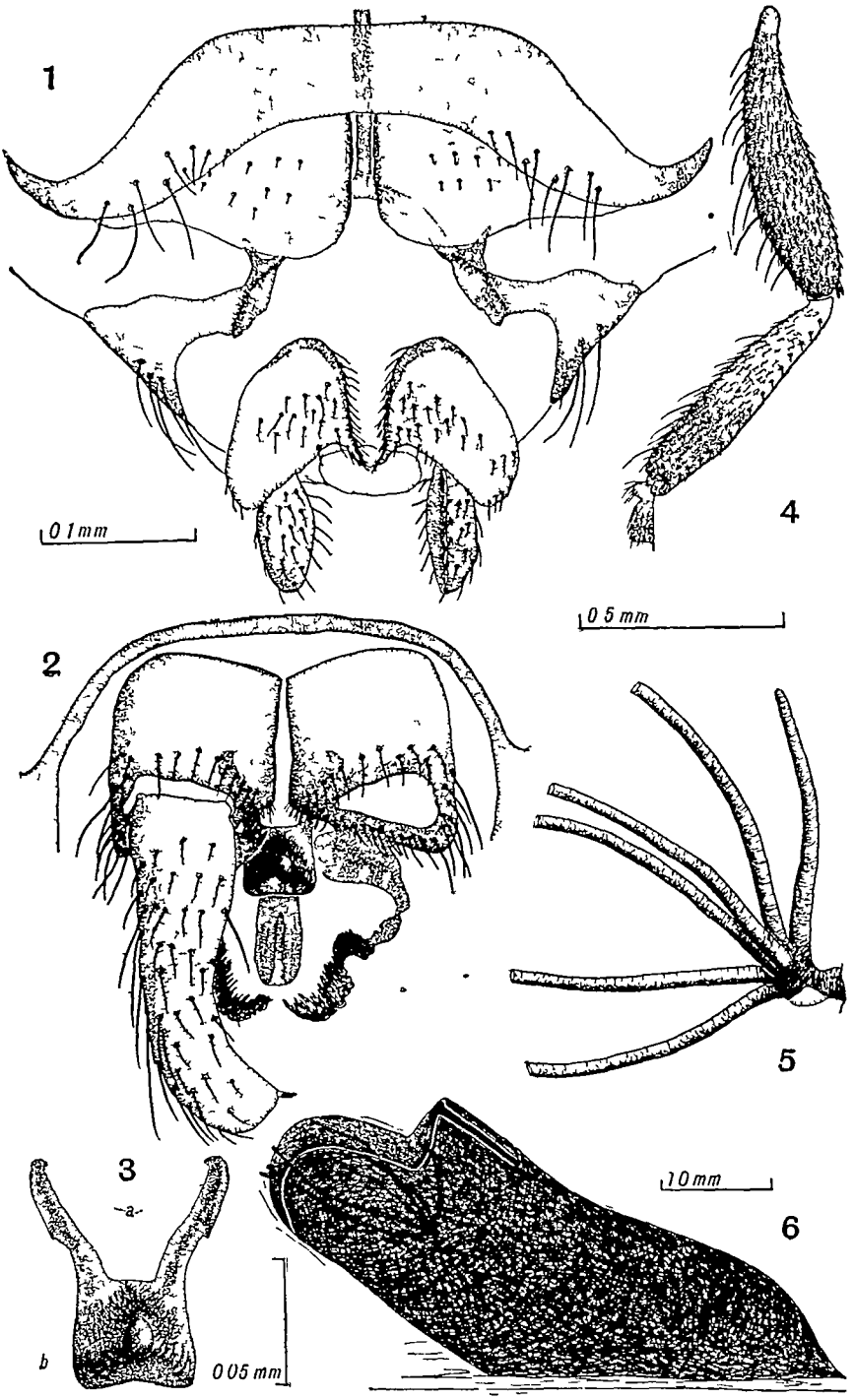
segments somewhat yellowish brown in their proximal halves and with very fine whitish pubescence as usual Palpi black

Thorax—Mesonotum velvet black, densely covered with coarse golden pubescence In the fore corners are a pair of large, iridescent, silvery spots (with a slight bluish sheen), broadly separated in the middle The whole of these spots reflects light at one time In certain lights the mesonotum shows a broad silvery border laterally and somewhat paler posteriorly and also a slight grey colour between the two anterior spots Scutellum is brown covered with coarse golden pubescence and has a fringe of long black hairs Pleuræ slate grey, membranous area bare

Abdomen velvet black with scattered fine golden pubescence and with the usual silvery spots on segments 2 and 5–7 , long hairs on the yellowish black basal scale golden black *Genital armature* closely resembles that of *S griseifrons* (Puri, 1932) The styles are long, being only a little less than four times its greatest width near the base Beyond the basal one-third, the styles become gradually narrowed and broaden out slightly again in the distal one-fourth On their dorso-internal surface near the base each of the styles bears a triangular protuberance directed slightly upwards, inwards and forwards towards the base These processes are strongly toothed along their free edge and appear comparatively smaller than those present in *griseifrons* The inter-coxal piece (Plate XXXV, fig 11) has a moderately broad base from which a short gradually narrowing tongue-like process projects downwards [In one of the paratype specimens in which the inter-coxal is not as distended as in the other the process is turned slightly forwards and it has a narrow distal end which is suddenly bent backwards again (Plate XXXV, fig 12)] The posterior surface of the process is smooth but the anterior and ventral bears irregular rows of minute setæ

Legs—Fore coxæ greyish yellow, posterior ones black , trochanters dark grey, somewhat yellowish basally , femora brownish black, somewhat yellowish at the base and nearly black near the distal end, the middle femora comparatively paler than the others Fore tibiæ black, practically without any grey spot on its outer surface, only slight greyish sheen basally , tarsi black, slightly flattened, the length of the first segment being about 7 times its greatest width near its distal end Segments one and three with the usual pair of long black hairs sub-terminally on their posterior border Middle tibiæ brownish black with a yellowish base and distal half to one-third black , first tarsal segment diffusedly yellowish black on the basal half, the rest of hind tarsi black Hind tibiæ black with a somewhat yellowish basal end , basal half of the first and the base of the second tarsal segment yellowish black, the rest of hind tarsi black Hind basitarsus (Plate XXXV, fig 13) moderately broad It is only a little shorter than the hind tibiæ (0 9 of the length of the latter) and its greatest width in its distal one-third is 0 85 of that of

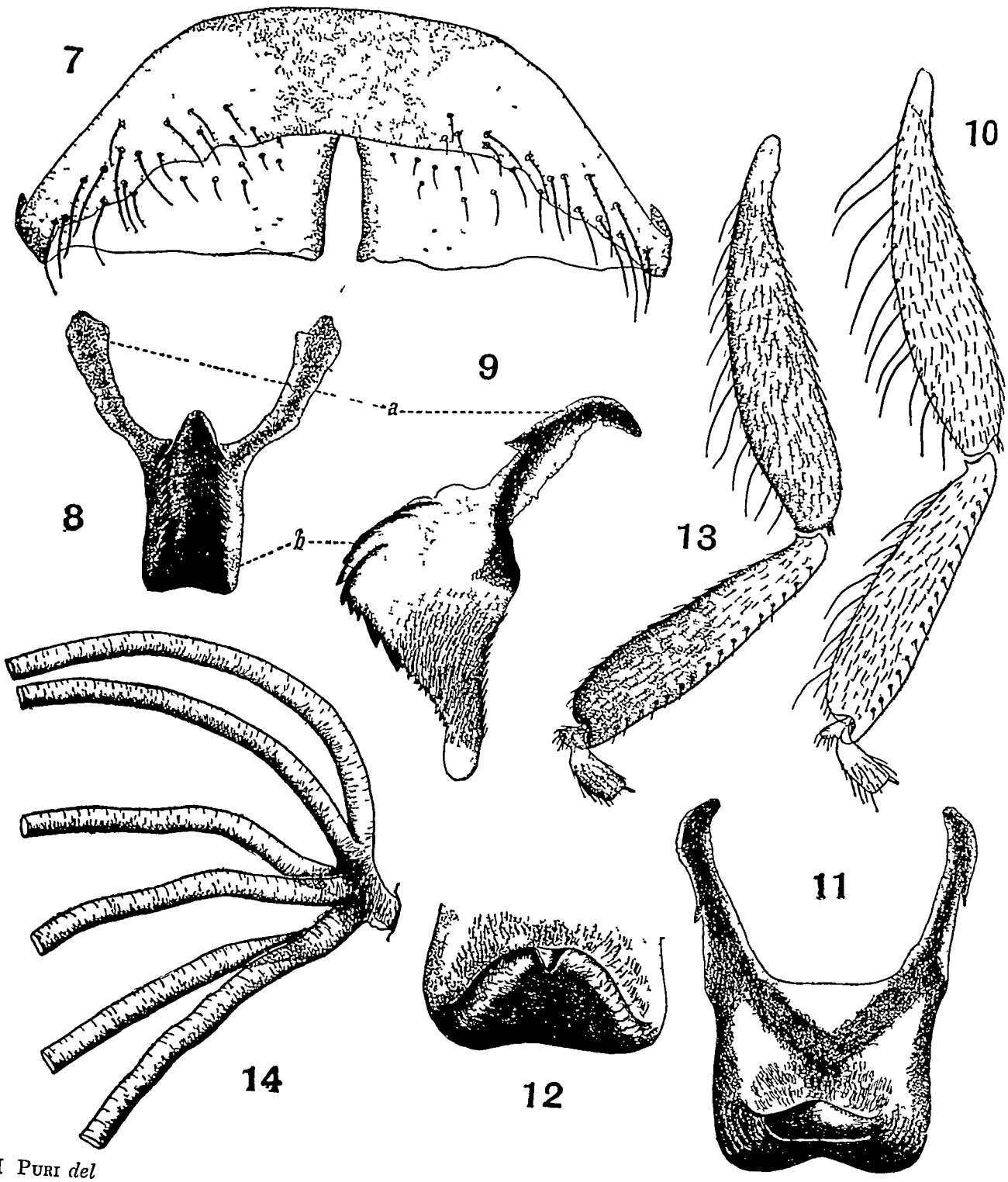
Plate XXXIV



I M Puri *del*

Simulium gravelyi sp n

Fig 1 Ventral view of terminalia of a paratype female
„ 2 Ventral view of genital armature of a paratype male Left style and cerci not shown Scale as in Fig 1
„ 3 Ventral view of inter coxal piece (*a*—apodemes, *b*—base)
„ 4 Tibia, basitarsus and 2nd tarsal segment of right hind leg of a paratype male
„ 5 Parts of pupal respiratory filaments of left side Scale as in Fig 4
„ 6 Lateral view of cocoon (pupa *in situ*)



I M PURI *del*

Simulium palmiense SP N

Fig 7 Sternite of segment 8 and anterior gonopophyses of a paratype female
" 8 Ventral view of inter coxal piece of a paratype male (*a*—apodemes, *b*—base)
" 9 Lateral view of inter coxal piece of a paratype male
" 10 Tibia, basitarsus and 2nd tarsal segment of right hind leg of male, showing the relative sizes of the various parts

Simulium tenuitarsus SP N

Fig 11 Ventral view of inter coxal piece of a paratype male
" 12 Ventral view of part of the inter coxal piece of another paratype male
" 13 Tibia, basitarsus and 2nd tarsal segment of right hind leg of a paratype male
" 14 Parts of pupal respiratory filaments of left side

(Scales as for figures of corresponding parts on Plate XXXIV)

the latter and about one-fourth its own length (Cf *griseifrons*)* Calcipala and pedisulcus both well marked Fine golden black pubescence mixed with black hair present on the legs, the latter predominating

Wings normal hyaline, radius hairy throughout its length, radial sector a simple, concave vein Wing length about 3 1 mm† Halteres yellow

PUPA

Size about 3 3×1 1 mm

The integument of the head and thorax is brown in colour, with disc-like tubercles scattered all over, those on the anterior three-fourths of the mesonotum comparatively large and closer set than on the rest The dorsal sub-median group of trichomes on the thorax is formed of three pairs so that there are five pairs of trichomes on the mesonotum dorsally These, as well as the three pairs of sensory hairs on the head, divide two or three times a little above their base, so as to form 7 to 9 long branches The cuticular hooks on dorsal as well as on the ventral surface of abdomen are as in *himalayense*, with a sensory hair somewhat strongly chitinized on the ventral surface of segment 4 Dorsally there is a row, broken in the middle, of the usual backwardly directed cuticular spines along the anterior border of segment 8, a few very small spines being present also on segment 7 Sub-terminal spines present on segment 9

Respiratory filaments (Plate XXXV, fig 14) are white in colour on their basal half and gradually become grey distally They are a little more than one-third the length of the pupa, six on each side, arranged in three pairs, the upper and lower of which have short stalks while the middle one is almost sessile The uppermost filament is directed upwards and forwards and the lower a little downwards and forwards, the rest of them spreading more or less uniformly in between them The filaments appear somewhat swollen in their basal half Their surface is broken up by transverse ridges which bear minute tubercles, resembling the arrangement found in *himalayense*

Cocoon is dirty brown in colour, boot-shaped, its opening directed upwards Its length from the posterior end to the anterior border of the opening is about 5 0 mm and that up to the posterior border (of opening) about 3 3 mm Its greatest width is about 1 7 mm The cocoon is very loosely woven, particularly in the anterior portion, to some extent approaching the condition found in *S striatum* (Puri, 1932*a*), though the mesh is comparatively closer than in the latter species

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* The hind basitarsus in *griseifrons* (male) is comparatively much broader and moreover uniformly broad practically throughout its length Its greatest width about the middle is a little more than one third its length [and not about half as erroneously stated in the original description of the male (Puri, 1932)]

† Wing length in type male of *griseifrons* is about 2 7 mm

Described from three males, two bred out of isolated pupæ, all collected from a large stream north of Marianbarie Tea Estate, Bengal Terai (March 1928)

Type and paratype in my own collection

This species closely resembles *S griseifrons* Brunetti but the lack of a conspicuous silvery white spot on the fore tibiæ, comparatively narrow fore tarsi and hind basitarsus, comparatively darker legs and the somewhat different form of the inter-coxal piece in the male and the fewer branches of the thoracic trichomes and the somewhat different nature of the respiratory filaments and the cocoon in the pupa at once distinguish it as a species quite distinct from the latter

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Ind Jour Med Res, XX, 3, *January*, 1933

STUDIES ON INDIAN SIMULIIDÆ

Part VII

DESCRIPTIONS OF LARVA, PUPA AND FEMALE OF *SIMULIUM NODOSUM* SP NOV, WITH AN APPENDIX DEALING WITH *S NOVOLINEATUM* NOV NOM (= *S LINEATUM* PURI)

BY

I M PURI, M SC (Punjab), PH D (Cantab), F E S,

Officer In-charge, Inquiry on the Indian Simuliidæ, Culicoides and other Blood-Sucking Midges

(*From the Central Research Institute, Kasauli*)

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AMONG a very large number of specimens bred out of isolated pupæ collected from the rushing waters of the Cauvery River near* Frazerpet (Coorg), a single female specimen was hatched out of a pupa, having somewhat peculiar respiratory filaments An examination of the adult shows that it possesses the characters of the sub-genus *Simulium*, as defined by Edwards (1931), i e, having the basal section of the radius bare, fore tarsi flattened, claws without a basal tooth and the tip of the abdomen shining The terminalia of the adult and the pupal respiratory filaments appear markedly different from those of any other species in this sub-genus described so far, but their characters are not such as to justify putting this species in a sub-genus separate from *Simulium*

Simulium (Simulium) nodosum SP N

FEMALE

Head black, with short black hairs on the occiput, a few present on the face and also along the lateral borders of the frons Frons black, distinctly shining,

* Under the bridge spanning the river just before the Mysore Mercara Road enters Coorg territory

comparatively wide, nearly one-third the width of the head, somewhat parallel-sided, being very little narrowed in the region of the antennæ Its greatest width near the top is about three-fourths its own length Face also comparatively broad, dull, silvery grey Antennæ brownish black, with the two basal segments yellowish brown, the dark portion with a fine pale pubescence Palpi black

Thorax—Mesonotum black*, markedly shining with a slight metallic lustre, sparsely covered with very fine short black hairs Scutellum black, with only a row of long black hairs, a few present also on the prescutellar area Pleuræ black, membranous area bare

Abdomen black, practically bare, fringe of hairs on the basal scale very short, somewhat golden black, second tergite with the usual greyish reflections, tergites of segments 6–8 very large, black, shining, sparsely covered with fine black hairs A small circular somewhat shining black tergite also seen on segment 3 *Terminalia* (Plate XXXVI, figs 1 and 2) The ventral surface of segment 7 bears short, widely separated simple hairs Sternite of segment 8 appears to be of a peculiar shape as shown in Fig 2 Its anterior border is broadly curved with long drawn out narrow lateral ends, while its posterior border is produced backwards on each side of the middle line as two broad processes directed a little downwards and inwards (Fig 1) These processes look like anterior gonopophyses but they do not show any line of demarcation separating them off from the main sternite and a thinly chitinized portion of the sternite appears to lie between the posterior process and the lateral end of the corresponding side Except for a small area in the middle, the sternite bears short macrosetæ more or less uniformly scattered on it and a cluster of stout and long ones near the distal end of the paired processes The anterior gonopophyses appear to be much reduced and are placed along the inner borders of the posterior processes of the 8th sternite They are thinly chitinized and bear only microsetæ on them The paraprocts are exceptionally large and strongly chitinized, bearing a cluster of fairly strong setæ at their posterior end The cerci are of moderate size.

Legs—Fore coxæ and trochanters yellow, femora brownish yellow, brown distally, tibiæ black, with a large silvery spot on the outer surface, the latter fairly conspicuous, tarsi black, flattened, the first segment about four times as long as its greatest width near its distal end, segments 1 and 3 with a pair of long black hairs sub-apically on their posterior border Middle and hind coxæ black, trochanters yellow, femora and tibiæ black, the former slightly pale at the base and the latter with a pale grey sheen on their posterior surface basally The first three

* In a specimen, belonging to this species, in the collection of the Imperial Agriculture Institute, Pusa, the colour of the thorax is slightly brownish but this is due probably to the specimen being somewhat discoloured as the head and also the black portions of the legs are brownish

tarsal segments of the middle leg and the basal three-fourths of the first and the basal halves of the second and third tarsal segments of the hind leg pale yellow, the rest of tarsi black Calcipala of moderate size, pedisulcus well marked All claws simple Yellow parts of legs with fine pale pubescence

Wings normal, hyaline radius bare up to the fork, radial sector a concave vein, bearing setæ on its ventral surface Wing length about 2 0 mm Halteres are pale lemon yellow

The posterior border (Fig 3) of the ventral surface of buccal cavity strongly chitinized and with a large number of short, close-set, spine-like projections in a cluster along its hinder border These spines are produced into the pharyngeal cavity The posterior arms of the furca are comparatively short and somewhat curved Spermatheca single, globular and uniformly dark brown as usual

PUPA

Size about 2 2×0 8 mm

Integument of head and thorax is brown, smooth and free from tubercles except on the posterior half or so of the thorax which is sparsely covered with very minute ones which are more or less spinous in form The trichomes on the head and thorax are very short and simple, the dorsal group on the thorax, composed of four pairs The cuticular hooks on the dorsal as well as on the ventral surface of the abdomen are as in *himalayense*, a pair of strongly chitinized hooks present on the ventral surface of segment 4 Dorsally on segment 8 there is a poorly developed row, broken in the middle line, of backwardly directed cuticular spines along the anterior border, a few small spines being also present on segment 9 The sub-terminal spines are absent

Respiratory filaments (Plate XXXVI, fig 4) are very short, three in number, much dilated, in the form of three clubs, the ventral longest of the three They are pale grey in colour The surface of the filaments is raised into poorly developed ridges forming a reticular pattern, very minute tubercles covering the interspaces and a little larger ones present on the ridges

Cocoon is dirty yellow in colour, of the ordinary wall-pocket shape, closely woven, without any interspaces or windows in the mesh, about 2 6 mm long and 0 9 mm broad near its open end

Described from a single female specimen, in fairly good condition, hatched out of an isolated pupa collected from the rushing waters of the Cauvery River near Frazerpet (Coorg), 9-1-1931, along with a large number of other pupæ belonging to *S striatum* Brun and *S grisescens* var *palmatum* Puri

Type in my own collection

In the collection of the Imperial Agriculture Institute, Pusa (Bihar, India), is a single female belonging to this species marked 'Pusa, Bengal, 30, iii, 1908 P E P.'

LARVA (full-grown)

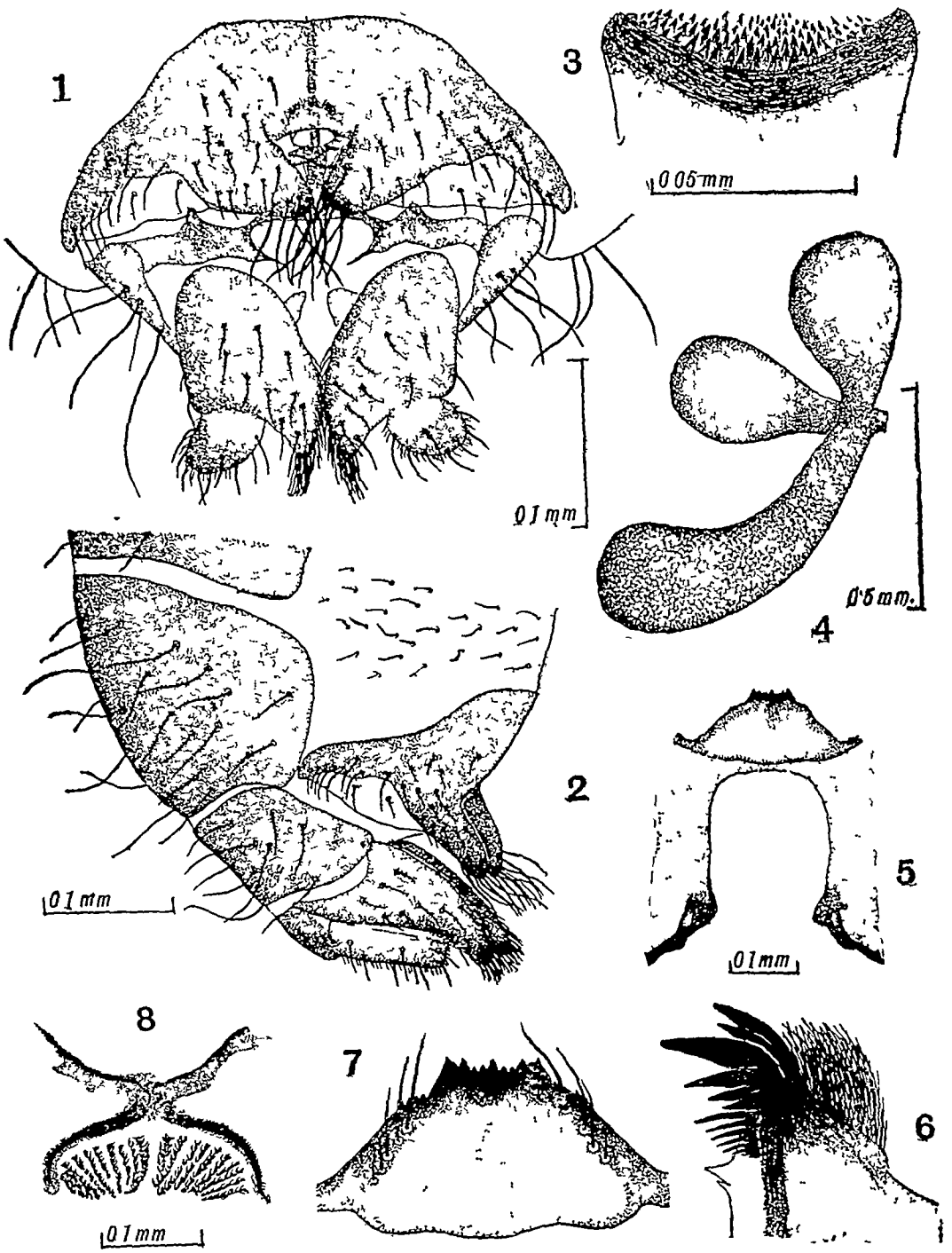
Length about 3 8 mm colour pale dirty brownish yellow

Head light golden brown, practically of a uniform colour, with practically no markings on it The poorly chitinized area on its ventral surface fairly wide, extending anteriorly nearly up to the sub-mentum (Plate XXXVI fig 5) *Antennæ* 4-segmented, basal segment about two-thirds the length of the second, which appears to be divided into two segments about its middle but the division is not complete, 3rd segment a little longer than the first Filaments forming the *cephalic fans* number 35 on each side *Mandibles* bear three small teeth in the sub-terminal row, and 7 spine-like teeth in the row a little proximal to the sub-terminal teeth The tooth-like process of the inner border, in the region of the spine-like teeth and the cluster of hairs behind the terminal teeth, are as in fig 6, Plate XXXVI *Maxillæ* of the usual form (Puri, 1925) *Sub-mentum* (Fig 7) has 9 teeth in the front row and a row of four on each side The median tooth and the one at each end of the front row are larger than the others, the third tooth from each end is the smallest in the row The posterior sucker has about 70 rows, each row having 10–13 hooks The anal chitinization is as shown in Fig 8 Ventral papillæ are not present at the posterior end

Described from a single full-grown larva, which was about to pupate, collected from the Cauvery River near Frazerpet, together with the pupa from which the female specimen described above was hatched out

In the adult stage this species closely resembles *S nitidithorax* and *S hirtipannus*, but besides the other distinguishing characters, its terminalia alone are quite enough to differentiate it from either of the latter two species The silvery spot on its fore tibiæ is not as bright as that in the latter two species In this character it appears to be intermediate between the species described in Parts I–IV of this study and those dealt with in Parts V–VI The females of the three species—*nitidithorax*, *hirtipannus* and *nodosum*, differ from all other Indian species belonging to the sub-genus *Simulium* in having a distinctly shining mesonotum, with a metallic tinge and with a sparse covering of very fine dark hairs These three species, which in general appearance look almost alike, can be differentiated very easily from one another by the following synoptic table —

1	Claws with a sub basal tooth, a large, median cluster of split hairs on the ventral surface of the abdominal segment 7	*S hirtipannus* Puri
	Claws simple, hairs on ventral surface of abdominal segment 7 simple and more or less uniformly scattered	2
2	Face black, shining	*S nitidithorax* Puri
	Face dull, dusted with ash grey	*S nodosum* sp nov



I M PURI *del*

Simulium nodosum SP N

Fig 1 Ventral view of terminalia of type female Fig 2 Lateral view of terminalia of type female Fig 3 Posterior end of ventral surface of buccal cavity, showing bucco pharyngeal armature Fig 4 Respiratory horns (filaments) of left side of pupa (Reticular arrangement of ridges shown on the lower filament only) Fig 5 Part of ventral surface of head capsule of larva showing the extent of the lightly chitinized area Fig 6 Ventral view of the end of (left) mandible of larva (Scale as in Fig 3) Fig 7 Dorsal view of submentum of larva (Scale as in Fig 1) Fig 8 Anal chitinization at the posterior end of larva

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APPENDIX

Simulium (Simulium) novolineatum NOV NOM

= *S lineatum* Puri, 1932

The name *lineatum* is preoccupied by Meigen's European species *Simulium lineatum* (=*Melusina lineata*, 1804) in which, according to Enderlein (1930), the fore-tarsi are not flattened and which belongs to his sub-family *Neveimanninæ* and the sub-genus *Wilhelmia* Consequently, I have re-named the Oriental species, *Simulium lineatum* Puri (1932), as *Simulium novolineatum* nov nom, which belongs to the sub-genus *Simulium*, with markedly flattened fore-tarsi

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PURI, I M (1932) *Ind Jour Med Res*, **19**, No 4, pp 1125–1143

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A YEAR'S OBSERVATIONS IN CALCUTTA ON THE INVASION OF THE SALIVARY GLANDS OF *ANOPHELES STEPHENSI* BY MALARIAL SPOROZOITES, AND THE INFLUENCE OF SOME CLIMATIC CONDITIONS

BY

C STRICKLAND, M A, M D (Cantab),
Professor of Medical Entomology,
D N ROY, M B, D T M (Cal),
Assistant Professor of Medical Entomology,
AND
H P CHAUDHURI, M B (Cal), D P H (Lond), D T M & H (Lond), F R S S (Lond)
(*From the School of Tropical Medicine and Hygiene, Calcutta*)

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MANY workers have recorded the presence of sporozoites in *Anopheles stephensi*, and we will refer below to some of their observations in conjunction with our own findings regarding the invasion of the salivary glands of this species during the course of a year's experimental work in Calcutta

MATERIAL AND METHODS

We used laboratory-bred mosquitoes and fed them on cases of malaria of any species of human *Plasmodium*

In these cases the gametocyte-count had usually been taken and shown at least 40 per c mm of blood, but in our earlier essays a count was not always made, reliance being placed on the gross appearance of a slide Counting was carried out by Sinton's standard fowl-cell suspension method, and in connection with this work we are much indebted to Dr B M Das Gupta, who has been kind enough to let us make use of those gametocyte counts that had been taken by him from our hospital in-patients We ourselves did the counts in those patients who attended

the out-patients' department, and appeared, on a preliminary examination of a blood-film, to have a sufficient number of gametocytes for our purpose

After the infecting feeds the mosquitoes were placed in cages in the laboratory, but otherwise they were kept under 'natural' conditions, being sustained on raisins and moistened lint only That the conditions in the laboratory inside the cages were not markedly different from those outside the cage, or the conditions of either site from those at the Bowbazar Meteorological Station, is seen from the following comparative figures taken by us within the period 3rd to 25th May, and one of us (H. P C) has shown that any differences in the temperatures were insignificant, and the Bowbazar* figures have therefore been mainly utilized for the correlations in his analysis of our infection-rates

Time of reading	Average temperature inside cage °F	Average temperature outside cage °F	Days	Bowbazar Station, average for same dates as laboratory readings °F
10—10 30 A M dry bulb	87 61	89 27	18	
wet ,,	79 4	78 72	18	
2 P M dry bulb	89 64	91 76	17	
wet ,,	79 52	78 29	17	
4 P M dry bulb	90 6	91 58	12	93 3
wet ,,	80 91	80 83	12	80 5

The 'dry bulb' reading was always a little higher outside the cage than inside, whereas the vapour-tension was always a little greater inside the cage, and the 'wet bulb' temperature therefore higher than outside

The first feed was given on 22nd January, 1931, and the last 29th February, 1932, and during this period 62 batches were fed

The least period that elapsed before we dissected any of the mosquitoes was 8 days, when the glands of 2 mosquitoes were found heavily infected The longest interval was 44 days when 4 mosquitoes were found sterile, 24 others, however, of the same batch, dissected between the 18th and 28th day, were also sterile The longest interval after which we found sporozoites in the glands was 41 days

Without analysis of our observations with regard to the species of parasite or any other particular circumstance† of our work, we found during the whole period

* The figures for 'mean humidity' that have been utilized by him were on the contrary obtained from the Alipore Observatory

† Except that the results are not inclusive of feeds when the gametocyte count was less than 40 per c mm (unless otherwise stated),

of our inquiry sporozoites in the salivary glands of 207 out of 664 mosquitoes, i e , in about 31 per cent, and we will now analyse these figures

PERIODIC VARIATIONS IN THE INFECTION RATE

TABLE I

Period	Examined	Glands infected	Rate per cent
22-1-31—7-2-31	46	42	91 3
10-3-31—18-7-31	393	3*	0 74
14-9-31—18-2-32	225	161	72 0

* A 'very scanty' infection in each case

We may therefore call the period March to July the off season, and the cold weather months the infective period Unfortunately owing to circumstances over which we had no control, we have no records for August and October, while September and February were liaison months

MONTHLY VARIATIONS

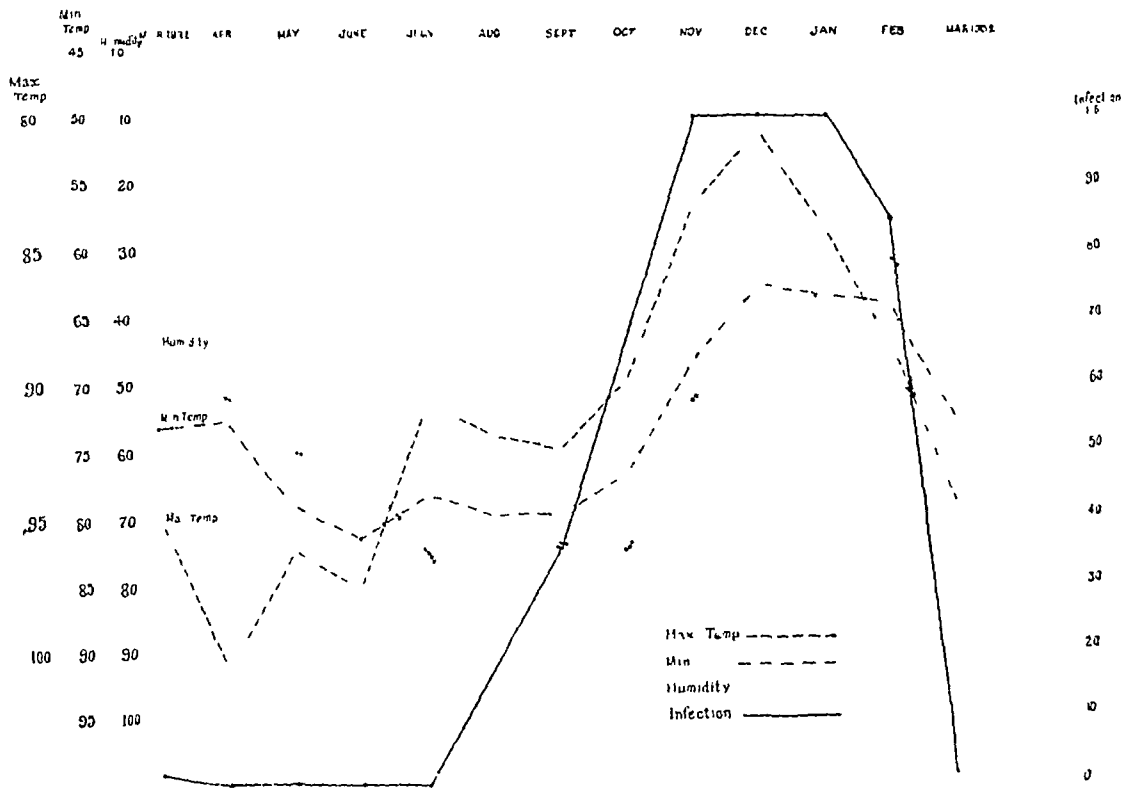
TABLE II

Seasons			Number of mosquitoes	Glands infected	REMARKS
'Infective season'	January	1931	15	11	3 noted as 'scanty'
	February	,,	31	31	
'Off season'	March	,	67	1	'very scanty' infections
	April	,,	37	0	
	May	,,	31	0	
	June	,,	130	2	'very scanty' infections
	July	,,	128	0	
'Infective season'	September	,,	61	27	13 noted as 'very scanty' infections
	October	,,			
	November	,,	29	29	6 noted as 'very heavy' infections
	December	,,	45	33	2 noted as 'very heavy' infections
	January	1932	59	57	
	February	,,	31	15	

The above periodic rates appear to point to the conclusion that the conditions of the Calcutta cold weather are optimal for the development of the parasites and that during the hot weather as well as the 'rains' there is a definite check to the invasion of the glands by the parasite (*see* Chart 1) The conditions favourable to the development of the parasite had a gradual onset in September, for not only was the rate of infection in this month intermediate in degree between the preceding and succeeding periods, but of the 27 mosquitoes that were then found infected, 13 were described as 'very scanty', this never having been noted in the 'infective season' when well-established Similarly at the other end of the 'infective season' the figures for February, in the two years 1931 and 1932 combined, showed a decline from the cold weather infection-rate

CHART 1

Inverse relationship of physical factors to infection



The infection-rate in *A stephensi*

The coefficient of correlation and other measurements between these infection-rates and the physical conditions prevailing has been worked out by one of us (H P C) whose note is included in this paper

This subject is discussed further on page 833

INFLUENCE OF GAMETOCYE-DENSITY IN THE INFECTING FEEDS

The greatest number of gametocytes that led to infection in the mosquitoes recorded in Table II was 1,200 per c mm and the least 40 per c mm,* the latter being on one occasion responsible for a 'very heavy infection'

For the purpose of the following analysis of the matter, we have utilized not only those data that relate to the mosquitoes fed on blood showing a gametocyte-count of at least 40, but in addition some other observations when the gametocyte-count was 'less than 40', and of this material we have only analysed that for the 3 months—November to January, when, as Table II shows, there was least variation in the infectivity-rate, so that the factor of season, so strongly indicated there, might be minimized as far as possible

The following table then shows the relationship between the infection-rates and the gametocyte-counts during this period —

TABLE III

Gametocyte counts per c mm	Examined	Infected	Infection rate per cent
480—1,200	43	43	100
120—180	37	37	100
40—80	55	39	71
Less than 40	35	11	31

It will be seen therefore that during the period of greatest infection (November to January), a gametocyte-count of 120 per c mm and over was sufficient to produce a 100 per cent infection, whereas with a count varying from 80 to 40 there was a decline in the infectivity, and below 40 a further decline

Accepting this view, the comparative depression in the December infection-rate, relative to that of November and January (*vide* Table II), may have been due to that rate partly arising from some mosquitoes fed on the blood of a case in which a gametocyte-count was not made and which may have been under 80, the mosquitoes fed on it showing only 1 in 11 infected The same consideration applies to the difference seen in Table II between the rates for January and February 1931, and the rates for the same months in 1932

* *See* also Table III where it will be noted that in a small number of mosquitoes, fed on cases with a gametocyte count of 'less than 40', infection also developed in the mosquitoes

THE INFECTIVITY OF THE SEVERAL MALARIAL SPECIES

During the 'infective' period, taken for this analysis to be from November to February, the following table shows the relative rate for each species of parasite —

TABLE IV

Parasite	Examined	Glands infected	Rate per cent
P falciparum	67	66	98
P vivax	119	102	86
P malariæ	24	9	37

During the 'off season', of the only 3 cases of infection that occurred, one was due to *P vivax* and two to *P falciparum*

The low rate demonstrated above for *P malariæ* may have been caused by the fact that, in one batch of mosquitoes fed on this species at the end of February, the non-infective period was being approached, and moreover the gametocyte-count was only 80 If one excludes any such equivocal cases, the above table becomes as follows —

TABLE V

November to January infection only

Parasite	Examined	Glands infected	Rate per cent
P falciparum	36	36	100
P vivax	51	51	100
P malariæ		'No Records'	

The analysis of the figures for *P malariæ* only is given in the following table Those obtained when the gametocyte-count was over 80, although they included observations in February, *at the approach of the off season*, gave an infection-rate of 82 per cent —

TABLE VI

Gametocyte count	Period	Rate per cent
180	November—February	82
80—180	November—February	37
80—180	November—January	No records

It therefore appears probable that the infectivity of the various species of parasite for *A stephensi* is maximal in each case

THE INFLUENCE OF CERTAIN CLIMATIC CONDITIONS ON DEVELOPMENT OF INFECTION

We know that mosquitoes, like most insects, die rapidly in a relatively dry atmosphere, though all mosquitoes are not affected in a precisely similar manner and even the anophelines differ in this respect considerably amongst themselves

Now while previous observers have thus shown that the life of mosquitoes is influenced by the atmospheric humidity, yet Gill (1911) has reported that 'humidity' has no *direct* effect upon the development of the malaria parasite in the mosquito, that any favouring influence this factor may have is due to its maintaining a favourable temperature, which on the contrary has a direct influence on the growth of the parasite

One must bear these facts in mind when he emphasizes the necessity of 'a high humidity' (not less than 60 per cent), for the propagation of the malarial epidemic, or when one considers the ancient belief that malaria is a disease of humid climates

As for the influence of *temperature* it is common knowledge that this is a very important factor in determining the geographical distribution of diseases in general and might be expected to be unexceptional in this respect in the incidence of malaria But the fact that the majority of diseases do not lie entirely within the defined zones of temperature, such as the tropics, is a clear indication that this factor plays a capricious part, that needs, in any particular case, careful analysis

The degree of the influence of humidities and temperatures in the matter of malaria incidence, from the point of view of the development of the parasite in the mosquitoes, has been the subject of the following analysis in which have been enunciated the values of some of the important factors concerned

The material in general from a statistical point of view had several defects, some of which may be mentioned —

(1) the observations were too few in number,

(2) they were not representative, because in the middle period between the highest and lowest infection-rates they were very scanty,

(3) the number of mosquitoes examined was not the same throughout the period,

(4) the temperature and humidity figures were those taken at a standard meteorological station and not in the laboratory where the experiment was being carried out, although the difference between the two places had no significant value Moreover at that station only the figures for 'minimum' relative humidity were obtainable

Such defects, however, were almost inevitable in the circumstances of the actual experiments For instance it was impossible sometimes to get cases of malaria showing gametocytes for the infection work

(a) The data for the whole period

After a long period of negative infection the positive infection recorded at the middle of September, gradually rose until by the middle of November it was cent per cent It continued around this figure* till the end of February when it began to come down and became (with the exception of 3 mosquitoes in 393†) absolutely negative from the beginning of March to the end of July

Of various factors possibly involved the influence of the following has been examined —

(1) maximum temperature,
(2) minimum temperature,
(3) relative humidity (4 P M),
(4) relative humidity (mean)

The variation in these factors over the whole period was as follows —

Maximum temperature	80° to 106°	mean	93 80°
Minimum temperature	62° to 86°	,,	75 36°
Relative humidity (4 P M)	20 to 100 per cent	,,	53 2 per cent
Relative humidity (mean)	45 to 95 per cent	,,	70 0 per cent

(b) Table of variation of infection, temperature and humidities at different seasons

		Season	Maximum temperature °F	Minimum temperature °F	Relative humidity (4 P M) per cent	Relative humidity (mean) per cent
1	Cent per cent infection	Nov to Jan	80—87	62—66	32 to 43	66 to 70
2	Infection of some degree	Sep to Feb	80—96	62—80	32 to 70	63 to 85
3	No infection‡	Mar to July	89—106	62—80	30 to 80	45 to 85

* Any discount probably being due to the gametocyte rate being too low
† 0 7633 per cent ± 0 296 (not significant)
‡ With the 3 exceptions noted above

From the above table it is clear that —

(1) infection took place at temperatures varying from 62°F to 96°F,

(2) infection took place at relative humidities ranging from 32 to 85 per cent,

(3) no infection was recorded above 96°F or below 62°F, and none above 85, or below 32 per cent relative humidity,

(4) the most favourable temperature for infection lay between 62°F and 87°F, or between the means of 71°F and 76 5°F

(5) the higher the maximum temperature above 81°F the lower was the infection rate,

(6) the higher the minimum temperature above 66°F the lower the infection rate

(7) the most favourable relative humidity (4 P M) was 32 to 43 per cent,

(8) the higher the relative humidity (4 P M) above 43 per cent, the lower the infection,

(9) the most favourable relative humidity (mean) was 66 to 70 per cent,

(10) the higher the relative humidity (mean) above 70 per cent, the lower the infection,

(11) the lower the relative humidity (mean) below 66 per cent, the lower the infection,

(12) the most favourable season for infection was from the middle of November to the end of January

(c) *Means and standard deviations of the data*

	Standard deviations	Means
Percentage of infection	36 16	23 8
Maximum temperature	7 27	93 8°F
Minimum temperature	7 01	75 36°F
Relative humidity (4 P M), per cent	17 28	53 20
Relative humidity (mean), per cent	12 15	72 00

(d) Coefficients of correlation

(A) Coefficients of correlation of infection with —

Maximum temperature	−0 7760±0 0368	Highly significant
Minimum temperature	−0 6826±0 0763	,, ,,
Relative humidity (4 P M)	−0 3379±0 0893	Significant
Relative humidity (mean)	−0 2620±0 0897	,,

The correlations have also been shown diagramically in Chart 2

(B) Correlations and partial correlations of the physical factors

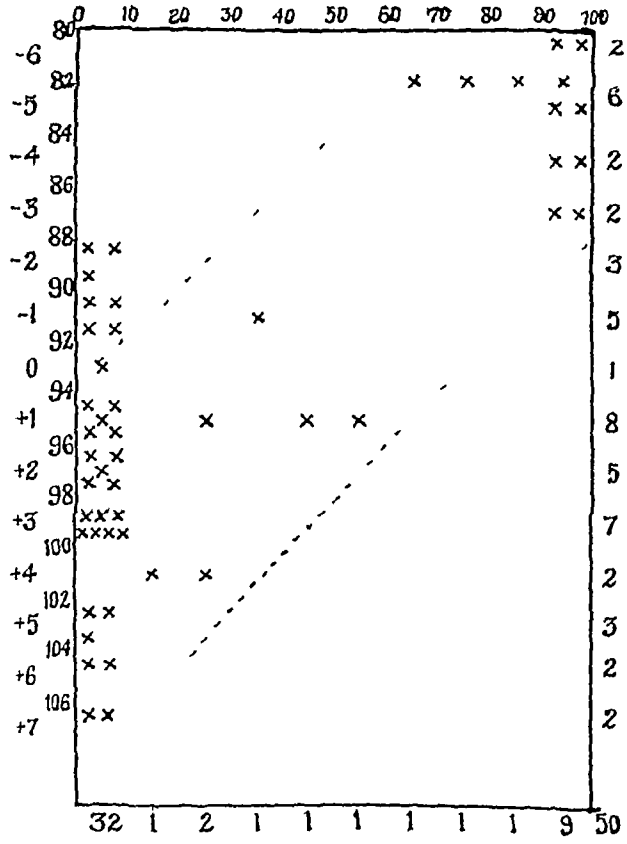
Relative humidity with minimum temperature being	+0 4507
Relative humidity with maximum temperature being and maximum temperature with minimum temperature being	+0 1863 +0 8795
Partial correlation of relative humidity with minimum temperature, when maximum temperature was constant, being ..	+0 6136
Partial correlation of relative humidity with maximum temperature, when minimum was constant, being	−0 4947
The partial correlation of infection	
(*i*) with relative humidity (4 P M)	
when maximum temperature was constant	−0 3120
when minimum temperature was constant	−0 5680
(*ii*) with maximum temperature	
when relative humidity was constant	−0 8930
when minimum temperature was constant	−0 5052
(*iii*) with minimum temperature	
when maximum temperature was constant	−0 0004
when relative humidity was constant	−0 5750
(*iv*) with relative humidity (4 P M.)	
when maximum and minimum temperature were constant	−0 3950±0 0889 (significant)

(C) Regression coefficients of infection in terms of —

Maximum temperature	−3 85
Minimum temperature	−3 55
Relative humidity (4 P M)	−1 418
Relative humidity (mean)	−0 779

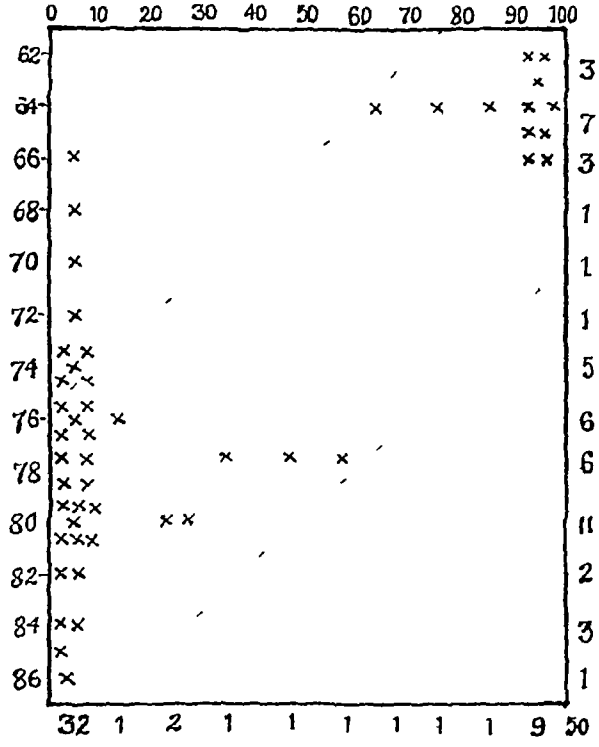
CHART 2

Percentage to Maximum Temperature



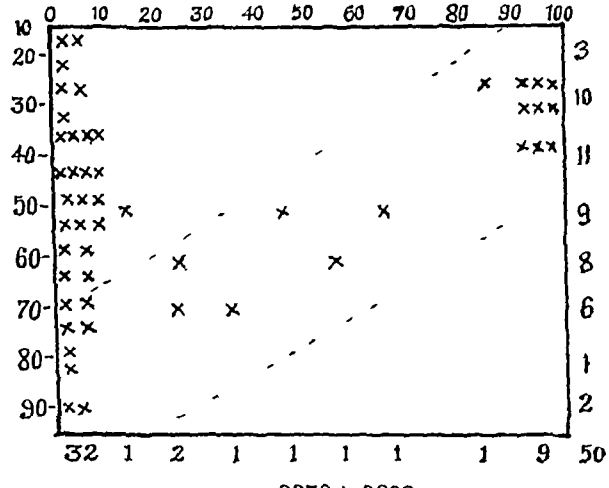
r=— 7760± 0568

Percentage to Minimum Temperature



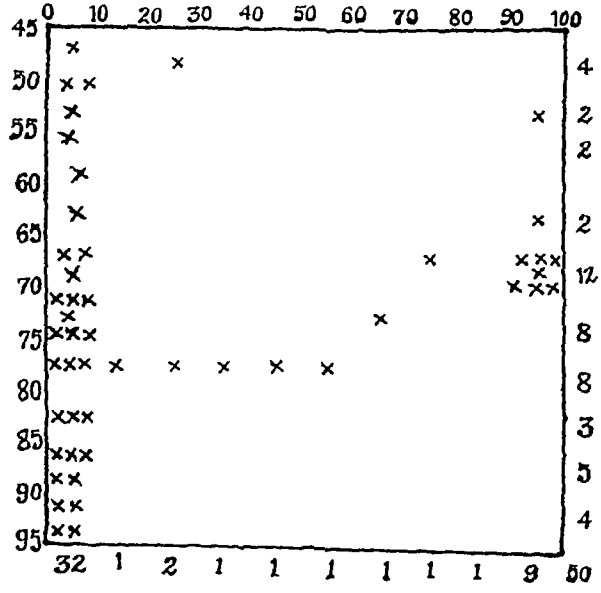
r=— 6826± 0763

Percentage to Humidity ('Minimum at 4 P M')



r=— 3379± 0893

Percentage to Humidity (Mean)



r=—·2620±·0897

Thus, we see that within the limits of the observations for every degree of rise of maximum temperature above 93 8°F , there was a 3 85 per cent decrease of infection and for every degree of minimum temperature above 75 4°F , there was a 3 55 per cent decrease

Similarly, there was less infection for every unit increase of relative humidity (mean) above 72 per cent

The coefficients show that the maximum temperature and minimum temperature, as well as the humidity, had a great influence on the infection of *Anopheles stephensi*, and that their importance in order was as follows —

(1) Maximum temperature
(2) Minimum temperature
(3) Relative humidity (4 P M) *
(4) Relative humidity (mean)

I The degree of influence of the various physical factors that were examined, on the infection rate of *A stephensi* has been shown in detail above , and the most interesting and significant of the findings was that *A stephensi* appears to react to humidity in a different manner from that reported by other authors In Calcutta the higher the relative humidity (mean) above 72 per cent, the less the infection , at 85 per cent the mosquitoes reach a complete freedom from sporozoite formation while Gill (1921) in the Punjab on the contrary found that the autumnal malarial mortality was positively correlated with the high humidity of July and August

II Gill (1921) said that the most favourable humidity for the transmission of malaria was 60 per cent or over, at a temperature of 78°F , as well as indirectly for the development of the parasite in the mosquitoes The above finding in this respect tallies with his remarkably, viz , that 66 to 70 per cent relative humidity with a mean temperature of 76°F to 71°F , was most favourable for the development of the parasite in its extra-corporeal stage

III Regarding low degrees of humidity Gill (1921) said that below 48 per cent and at 81°F , *Culex fatigans* infected with *P grassi* did not survive long enough to become infected In Calcutta on the other hand, while the relative humidity (mean) hardly ever went below 48 per cent, we have one case on the record, and in this it was 46 per cent, when 13 insects after feeding on infected blood survived for one week before dissection There were also several other cases in which the humidity (' minimum ', recorded at 4 P M) was less than 48 per cent, in which *Anopheles stephensi* survived long enough to complete the development of sporozoites Gill (1921) also said that epidemics in the Punjab never occur

* 8 A M readings are not reliable because the atmospheric condition prevailing at the time are subject to considerable variations due to the shifting of the time of sunrise from season to season

when humidity is *abnormally* low and every epidemic is associated with *high* humidity, though *extremely high* humidity is not followed by increased malarial incidence

In this respect also the statistical result here corroborates his statement, if we take 'abnormally low humidities' as those below 60 per cent, and extremely high as above 85 per cent

IV Regarding the temperature factor only, Gill (1921) concluded that in India malarial mortality was negatively correlated with a temperature above 90°F, whilst in England a temperature above 60°F exhibits a positive correlation with malaria, that is 60°F to 90°F was the optimum range of temperature for transmission

The above analysis to some extent confirms this, because infection actually occurred within the range of 62°F to 96°F, though it was negatively correlated with temperatures above 80°F

V The analysis of the data and the partial correlation table shows to what extent the variation of temperature is relevant regarding the infectivity of Anopheles, or in other words the relative importance of the factors that act alike upon the sporozoite infection, compared to the totality of factors at work When we know that a phenomenon is probably directly influenced by certain physical factors, then the calculation of the partial correlations gives a most valuable account of the causation of the phenomenon The results of the analysis may be put in the following way —

Relationship of relative humidity with infection

(1) When all the physical factors are varying	—0 3379
(2) When maximum temperature remains constant	—0 3120
(3) When minimum temperature remains constant	—0 5678
(4) When both maximum and minimum temperature remain constant	—0 3950

We know that all these coefficients are significant for a sample of 50 The relationship is most marked when the minimum temperature remains constant and least when the maximum temperature remains constant

Thus, from the statistical analysis there can be no doubt about the direct importance of the relative humidity on the development of the parasites, though Gill (1921) has found otherwise in his experimental observations The glandular infectivity of *Anopheles stephensi* diminishes with an increase of humidity above 72 per cent and increases with its decrease below 72 per cent

VI To sum up it may be said that —

(1) the optimum range of infection lies between 66—70 per cent relative humidity (mean), and 71°F —76°F temperature (mean),

(2) an unfavourable range of infection lies above 85 per cent and below 45 per cent relative humidity (mean), as well as above 95°F and below 65°F temperature

VII From the results of this analysis, it is evident that *Anopheles stephensi* in Calcutta should not carry any malaria at all in a season when the relative humidity (mean) figure is above 85 per cent or below 45 per cent and when the temperature goes higher than 95°F or drops to 62°F or less, i e, conditions represented within the period of April to October

SUMMARY

(1) Examination of the sporozoite infection of the salivary glands of *Anopheles stephensi* in Calcutta in 1931 under experimental conditions has shown a very striking period of infection from November (October ?) to January (90 per cent) and an equally striking period of non-infection from March to July (August ?) (0 7 per cent), with transitional conditions in September (44 per cent), and in February (74 per cent)

(2) The two main periods corresponded roughly with (*a*) the cold-weather, and (*b*) the hot-weather

(3) Chart 1 indicates plainly that the infection was inversely correlated with the maximum temperature, and, but not so markedly, with the minimum temperature, but the influence of relative humidity (minimum) on the infection was not clear

(4) A gametocyte-count of 120 per c mm of blood, or over, had a potential infectivity of 100 per cent Below this density there was a gradual decrease in infectivity

(5) Both *P vivax* and *P falciparum* had a 100 per cent infectivity *P malariæ* had, within the limits of our observations an 82 per cent infectivity, but we believe it probable that it does not vary from the other species in this respect

(6) (*a*) There have been stated the limits of the physical conditions within which there was cent per cent infection, some degree of infection, or none at all The correlations involved have also been calculated, and then it was discovered that, not only the maximum and minimum temperatures, but also the relative humidities (both the mean and the minimum) had a significant and inverse influence on the infection-rate

(*b*) The coefficients showed that the maximum temperature, minimum temperature, and relative humidities (both minimum and mean), were influential in this order

(*c*) The partial correlation of infection with humidity (minimum) when both maximum and minimum temperatures were constant, *showed a significant value for the influence of humidity*

(*d*) Above and below each datum scheduled below, there was a respective decrease and increase of infection by the amount noted in the last column —

TABLE VII

	Datum	Regression in infection rate Per cent
Maximum temperature	93 80°F	3 85
Minimum temperature	75 36°F	3 55
Relative humidity ('minimum')	53 20 per cent	1 418
Relative humidity (mean)	72 00 per cent	0 779

DISCUSSION

Although a large number of workers have stated the rate of malaria-infection, both 'natural' and experimentally induced, found in anopheline species, and *A stephensi* has had a share of this attention, comparatively few reports have appeared regarding the relationship of the infection to the physical conditions prevailing at the time of the observations, while, of those workers who have made any, we believe that no one has taken out the coefficients of correlation of the infection and the physical factors as has been here done With such observations however, as have been made, we will now attempt to compare the results summarized above

Regarding *A stephensi* Bentley* (1911) in Bombay showed that, of all those he dissected during a year and more, 83 per cent found infected were obtained during the months of highest relative humidity (July to September) On the other hand it seemed that either extreme heat or low temperature inhibited infection

Now it will be noticed that his findings—his mosquitoes were 'naturally' infected—were the converse, one might say, of ours experimentally obtained in Calcutta While in July (1910) he obtained a 10 per cent rate (a very high 'natural' rate), we found experimentally in the same month 0 per cent, and while he found 83 per cent of all his sporozoite-infected mosquitoes in the three months of the 'rains', July to September, we obtained in these months only about 13 per cent We therefore appose to his conclusion that the relative humidity was the most important positive factor in his findings, the corresponding gross result of our analysis that there was within the limits of the Calcutta observations an

* Bentley's data are given in an Appendix to this paper

inverse relationship between infection and humidity, a finding confirmed on taking out the coefficient of their partial correlation

Regarding the influence of extreme heat on infection If the limits of maximum temperature (89°F —106°F) within which we found absolutely no infection in Calcutta, may be designated ' extreme heat ', then we agree with Bentley's conclusion that infection does not occur under conditions of ' extreme heat ' But regarding the influence of ' low temperature ' which he also thought inhibited infection, if the limits (62°F —66°F) within which we found cent per cent infection, be considered to include ' low temperatures ', we cannot agree with him We had, however, no opportunity to examine the matter at any lower temperatures than 62°F and perhaps Bentley referred to such lower temperatures

As it seemed to us to be so important to try to reconcile the differences noted above between Bentley's observations and our own, differences that might be more apparent than real, we have analysed his infection-rates with the coincident meteorological data, as given in his report (*see* Appendix), and have found that —

(*a*) On charting his monthly data there was an apparent positive correlation between the relative humidity rates (mean of mean daily observations, 1873—1896)*, and the natural sporozoite infection-rates Also one of us (H P C) has worked out the coefficient of correlation in his data and found it to be +0 8914±0 88 and therefore highly significant, which confirms the view that his and our own experiences were widely divergent,

(*b*) His figures for maximum temperature and minimum temperature were not apparently related to the infection-rate, the coefficients of correlation were respectively +0 144±0 192, and +0 3145±0 184 This also was contrary to our findings in Calcutta (*see* Chart 1 and the analysis of our corresponding data showing highly significant inverse correlation),

(*c*) The limits of variation, within which *some* degree of infection was found, were as follows, our own findings being placed in apposition —

TABLE VIII

Findings	Period	Maximum temperature °F	Minimum temperature °F	Relative humidity (mean) Per cent
Bentley's	July—January	83—87	67—79	71—89
Ours	September—February	80—96	62—80	63—85

* The figures for the monthly mean relative humidity, compiled from the weekly relative humidity records over the actual period of Bentley's observations, closely corresponded with those data

This table does not show a great difference between the circumstances of the occurrences at Bombay and Calcutta, and is chiefly noticeable for the fact that in Bombay the limits in the conditions in which any infection was found were considerably narrower than in the case of the experimental infection in Calcutta

In general then Bentley's findings are shown to be the converse of ours, though there was not a great difference in the two cities between the limits of variation in the conditions in which some infection took place

Now in view of the divergences noted, we are left to conclude either that the development of the sporozoites in *stephensi* in Bombay is determined by different conditions from those at work in Calcutta, or that there is no difference in this respect and that our respective experiences were merely diverse expressions of the same phenomenon biassed by other factors

On the evidence before us there is no immediate means of arriving at the truth, but we are inclined to believe in the latter alternative Bentley himself says that 'in periods of heavy rain' the sporozoite-rate decreased probably because the mosquitoes were 'drowned' The fact that in the two places the limits of variation (as shown above), in the conditions within which there was any infection, were not considerably divergent, is an indication that the mosquito is only a culture tube, in which the parasite reacts in a standardized way to its environmental conditions, and that, if in 'natural' infections there be found any differences in the infection-rate, they are but local expressions of the play of various factors on the mosquito In other words simple correlation has only a local significance, for instance our own finding in Calcutta of an inverse influence of humidity on the infection, Bentley's of a positive correlation *in the corresponding data*, Bruce-Mayne's (1927) at Saharanpur, or Gill's (1921) experience in the Punjab

We reiterate that one of us (H P C) has discovered a significant relationship between humidity and infection, while Jansco (1904), who earns our particular respect for attempting to put this important subject on an exact basis so long before his successors, thought that humidity, at a range of temperature between 64°F to 79°F, had no direct effect on the development of the parasite and if this thesis be true it would only be confirmatory of the opinion of Buxton and others that the physical conditions in the tissues of an insect do not necessarily represent the conditions of its environment The degree of humidity in the tissues is doubtless kept within narrow limits by physiological processes, so that it would not be remarkable to find that wide variations of atmospheric humidity would have but little effect on the development of a parasite in those tissues However if humidity is, then the temperature also may be, regulated by vital processes, though in this case some simple phenomena

like hibernation show that the regulation must be limited, and if so, there is no reason to suppose that humidity is any better controlled We may therefore conclude that both temperature and humidity may influence the development of the parasite

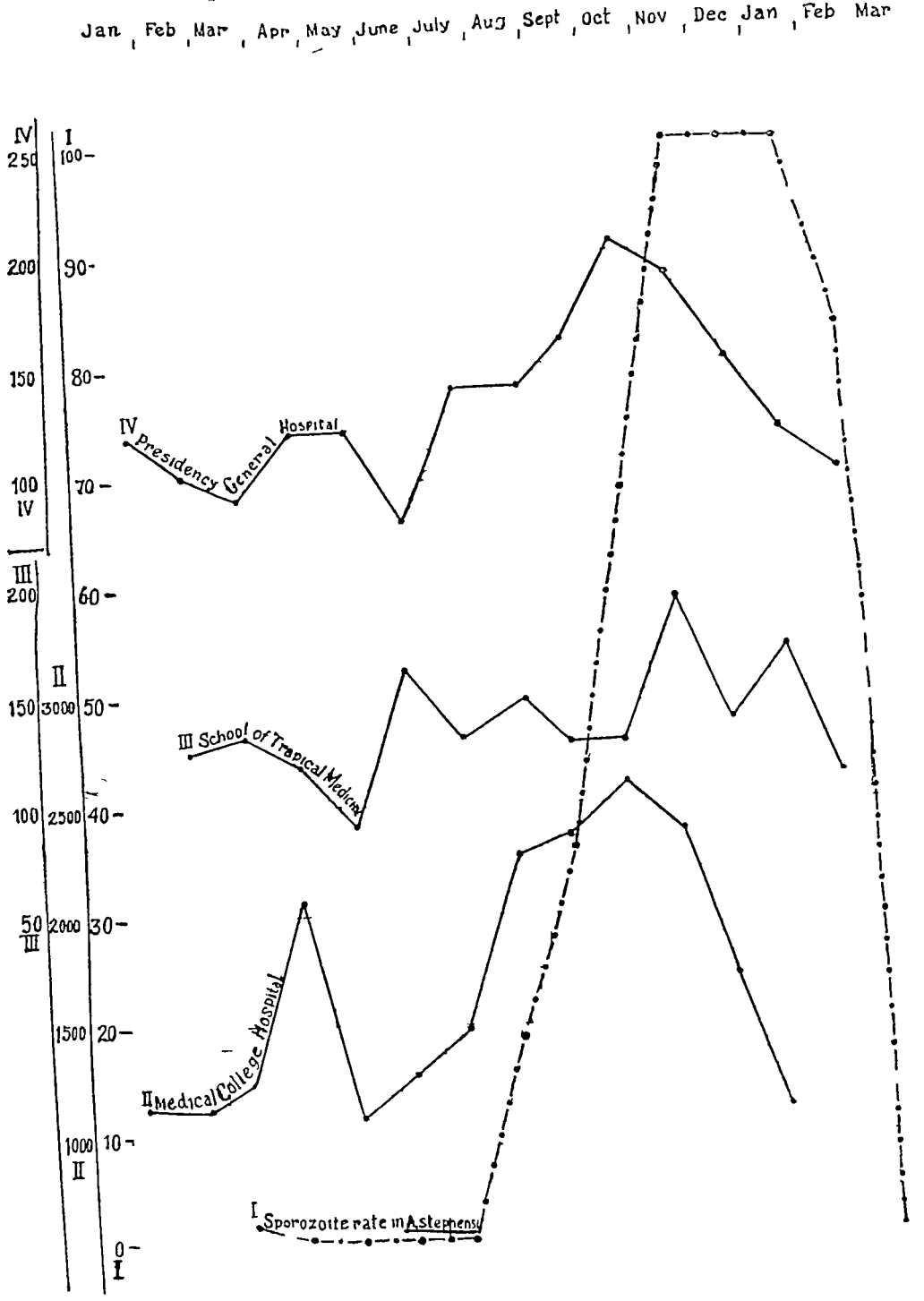
If we accept the theory, that the developing parasite in mosquitoes of all species reacts in the same way to the physical conditions of their environment, we must remark that, in view of the very diverse practical experiences of many observers, any correlations with the local physical conditions, that one may discover, do not necessarily denote any direct influence of any of those conditions on the parasite The only valid methods then of determining the influence of any physical factor are (1) the experimental and (2) the determination statistically of the ' partial correlation ', and the one method should be used to confirm the other

The direct influence of any particular factor on the development of the parasite, may of course be obscured, in the epidemiology of a disease, by antagonistic factors Hence it is not surprising that, though in the present enquiry we have concluded that in Calcutta the optimum conditions for the development of the parasite are represented within the period of November to March, there are no apparent consequent effects on Calcutta's health Dr Coltman has kindly given us the monthly malaria incidence record of the Presidency General Hospital for the years 1920—1931 , we also have the out-patients records of our own Hospital for the year February 1931 to February 1932 and also the Medical College Hospital figures for the corresponding period The incidence is shown in Chart 3 The slight monthly variations in incidence do not appear to have any relationship to the coincident variations in the infection-rate That is to say the operation of the primary factor has been biassed by other factors Thus also Soesilo (1928) in Java found *A vossi* infected 100 per cent and causing no local malaria, and thus also Covell (1927) remarks with sorrow that *A tankhudi* had been incriminated as an epidemiological carrier on the strength of its experimental infection Such occurrences must be thought of before jumping to the conclusion that because an infected mosquito has been found anywhere, therefore a locality is in danger They should also be an object lesson to those who do not realize that epidemics do not depend on one factor only but on a host of factors and that not one link only must be present but all the links in the chain

It is to be hoped, that our conclusion, that the relationship between the development of the parasite in the mosquito and the physical conditions is invariable from place to place, may be confirmed and that it may lead to more economical sanitation programmes in the future

CHART 3

Sporozoite-rates and hospital malaria incidence



Conclusion

We are led to believe, from our analysis of some of the physical conditions under which proceeds the development of the malaria-parasite in *A stephensi* in Calcutta, and in other species according to the data of other workers, that that development has a fixed relationship to those physical conditions, and that of these humidity is *not* negligible, while temperature is important too high a temperature completely inhibiting infection

Now whatever may be such an immutable influence of any particular factor on the infection-rate of the mosquito, the operation of that influence must be biassed from locality to locality by other factors, so that we find in effect varying correlations between any factor and the infection in other words whatever absolute value a factor may have its consequence may be negligible, so that one should beware of drawing hasty conclusions from any such absolute value as to its practical importance For example *although in Calcutta the absolute influence of humidity on infection has been found to be inverse and significant*, yet here an inverse correlation between these two principals was discovered and in Bombay a positive correlation, and while in Calcutta in the cold weather we found a cent-per-cent infectivity of *A stephensi*, the commonest anopheline here, this high rate was not reflected in the incidence of malaria according to the records of 3 hospitals for 10 years

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Appendix

Dr Bentley's data

The result of the examination of 1,217 salivary glands of *Neocellia stephensi* is given below —

Month	Number dissected	Number infected	Percentage infected
1909			
July	63	1	1 5
August	70	4	5 7
September	56	2	3 5
October	14	1	7 1
November	18		
December	11		
1910			
January	6		
February			
March			
April	11		
May	14		
June	15		
July	90	9	10 0
August	158	5	3 1
September	79	4	5 0
October	94	2	2 1
November	60	1	1 6
December	67		
1911			
January	153	1	0 6
February	173		
March	35		
Total	1,217	30	2 4

Monthly mean values of relative humidity, and maximum and minimum temperatures during the year 1910 *at Bombay, compared with the Calcutta figures* (1931)

Month 1910	Bombay records 1910			Calcutta records		
	Relative humidity Per cent	Maximum temperature °F	Minimum temperature °F	Monthly means of temperature Bowbazar, 1931		Monthly means of relative humidity (4 P M) Per cent
				Maximum °F	Minimum °F	
January	71	83 7	67 3	83 25	66 70	73 25
February	66	86 3	69 1	86 04	69 08	67 00
March	71	87 8	73 1	88 30	73 60	70 50
April	75	90 2	77 2	90 2	77 1	73 50
May	76	91 3	80 2	91 22	80 20	76 00
June	84	87 0	79 3	86 50	79 15	77 25
July	82	85 6	78 8	85 80	78 90	82 75
August	87	83 7	77 2	83 74	77 16	88 80
September	87	83 8	76 7	84 05	76 52	89 00
October	81	87 3	75 8	86 67	75 70	82 75
November	71	85 5	71 8	86 14	71 80	71 20
December	74	84 1	69 0	84 17	68 92	77 50

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EXPERIMENTAL INFECTION OF ANOPHELINE MOSQUITOES

BY

M O T IYENGAR,

Medical Entomologist, Travancore

(*From the Malaria Research Laboratory, Bengal Public Health Department*)

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DURING 1930 and 1931, the author carried out a series of malaria infection experiments on laboratory-bred anopheline mosquitoes Gametocyte carriers for these observations were selected from among patients attending the Field Treatment Centre at Singur, Hooghly District, Bengal For these experimental feeds, only such gametocyte carriers were selected as did not show any mixed infection during two consecutive examinations at an interval of 4 days After the experimental feed on the selected gametocyte carrier, the fed mosquitoes were separated from the unfed ones and were kept in cages in the Laboratory at Calcutta The mosquitoes were given only a single infective feed after which they were kept on raisins and water They were dissected after an interval of 8 to 15 days from the date of feed In the series of observations reported in this article, several species of *Anopheles* were experimentally fed on gametocyte carriers of tertian, subtertian and quartan infections respectively The author is much indebted to the Director of Public Health, Bengal, for facilities offered while conducting this investigation

Usually, mixed batches of mosquitoes comprising two or more species of *Anopheles* were fed on the same gametocyte carrier In judging the susceptibility of the different species of *Anopheles*, the results of those experiments, in which none of the mosquitoes fed on the particular gametocyte carrier developed an infection, have been ignored It is probable that, in experiments in which none of the different species took the infection, the gametocyte carrier was not infective to the mosquito or that some other factors prevented the development of the infection in the mosquito Only those experiments are being considered here in which one or more individuals of any of the species of mosquitoes in the mixed batches took the infection from the particular gametocyte carrier

Observations were made on the following species of *Anopheles* —

(*a*) *A aconitus* Don,
(*b*) *A barbirostris* Wulp,
(*c*) *A culicifacies* Giles,
(*d*) *A fuliginosus* Giles,
(*e*) *A hyrcanus* var *nigerrimus* Giles,
(*f*) *A jamesi* Theob,
(*g*) *A ludlowi* var *sundaica* Rodenwaldt,
(*h*) *A maculatus* Theob,
(*i*) *A majidi* Young and Majid,
(*j*) *A minimus* Theob,
(*k*) *A pseudojamesi* Str and Ch,
(*l*) *A stephensi* Liston, and
(*m*) *A varuna* Iyengar

The results of the infection experiments are discussed below —

(*a*) *Anopheles aconitus* Don

Two infection experiments were carried out with this species, one on a subtertian carrier and the other on a tertian carrier The number that survived was small and in each of the two experiments only one specimen was dissected

Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
					Gut		Gland		Total	
					Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
					P falciparum					
147	782	Muchiram	31-1-31	11-2-31	1	1	1	1	1	1
					P vivax					
144	450	Panchi	21-1-31	3-2-31	1	1	1	1	1	1

The results show that *Anopheles aconitus* could be infected experimentally with *P falciparum* and with *P vivax* In both experiments sporozoite infection of the salivary glands was observed in 11 to 12 days Although the number of observations with this species is small, the positive results obtained in these experiments show that this species is experimentally a good transmitter

(*b*) *Anopheles barbirostris* Wulp

Three infection experiments were carried out with this species on carriers of tertian, subtertian and quartan infections

Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
					Gut		Gland		Total	
					Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
				P falciparum						
153	1049	Habib	7-2-31	18-2-31	2	0	2	0	2	0
				P vivax						
155	1137	Sufal	11-2-31	25-2-31	2	0	2	0	2	0
				P malariæ						
169	1954	Gokul	14-3-31	23-3-31	1	0	1	0	1	0

In all the three experiments the results were negative It should be admitted, however, that the number of observations made with this species is small

(*c*) *Anopheles culicifacies* Giles

Two infection experiments were carried out with this species

Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
					Gut		Gland		Total	
					Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
				P falciparum						
172	2056	Kamala	18-3-31	27-3-31	5	0	5	0	5	0
				P malariæ						
173	2349	Sudhir	25-3-31	4-4-31	2	1	2	0	2	1

The results in the infection experiment with *P falciparum* were negative In the experiment with *P malariæ*, oocysts were observed in one out of two specimens examined

(*d*) *Anopheles fuliginosus* Giles

A fairly large number of observations was made with this species The records are furnished below —

P falciparum

Serial number	Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
						Gut		Gland		Total	
						Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
1	118	10064	Kalicharan	29-10-30	12-11-30	2	0	2	1	2	1
2	149	814	Milan	31-1-31	11-2-31	5	1	5	0	5	1
3	150	927	Kamaladasi	4-2-31	14-2-31	11	3	11	0	11	3
4	153	1049	Habib	7-2-31	18-2-31	25	9	25	0	25	9
5	172	2056	Kamala	18-3-31	27-3-31	1	1	1	0	1	1
6	185	2960	Banku	15-4-31	27-4-31	3	3	3	0	3	3
7	193	4022	Guiram	16-5-31	30-5-31	3	0	3	0	3	0
8	198	4014	Renu	16-5-31	30-5-31	3	1	3	0	3	1
9	212	5070	Sisubala	20-6-31	3-7-31	1	0	1	0	1	0
10	230	6742	Nalini	1-8-31	10-8-31	4	1	4	0	4	1
					Total	58	19	58	1	58	20

Ten infection experiments were carried out with *A fuliginosus* by feeding the mosquitoes on *P falciparum* carriers out of which positive results were obtained in eight experiments Out of 58 specimens dissected after the experimental feed 19 were positive for oocysts and one for sporozoites in the salivary glands Oocyst infections were more frequent than sporozoite infections In many instances heavy oocyst infections were observed and in two instances mature oocysts were seen bursting under the microscope

P vivax

Serial number	Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
						Gut		Gland		Total	
						Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
1	139	19	Upendra	7-1-31	19-1-31	4	1	4	0	4	1
2	144	450	Panchi	21-1-31	3-2-31	4	1	4	1	4	1
3	145	598	Kristo	24-1-31	3-2-31	1	0	1	0	1	0
4	155	1137	Sufal	11-2-31	25-2-31	2	0	2	1	2	1
5	157	1209	Jaboona	11-2-31	23-2-31	5	0	5	1	5	1
6	158	1409	Savithri	18-2-31	2-3-31	11	0	11	3	11	3
7	159	1389	Umapada	18-2-31	2-3-31	10	2	10	0	10	2
8	195	3965	Jitendra	13-5-31	25-5-31	2	0	2	0	2	0
9	226	6255	Lakshmi	22-7-31	12-8-31	1	0	1	0	1	0
					Total	40	4	40	6	40	9

Positive results were obtained in six out of nine experiments with *A fuliginosus* fed on *P vivax* carriers Nine specimens were positive out of 40 specimens examined and six out of the nine specimens had developed a sporozoite infection of the salivary glands

P malariæ

Serial number	Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
						Gut		Gland		Total	
						Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
1	173	2349	Sudhir	25-3-31	4-4-31	1	0	1	0	1	0
2	218	5496	Banamali	1-7-31	16-7-31	1	0	1	0	1	0
					Total	2	0	2	0	2	0

The negative results in the *P malariæ* experiments are not of much value owing to the small number of observations

(e) *A hyrcanus* var *nigerrimus* Giles

A fair number of observations was made with this species The results are furnished below —

Serial number	Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
						Gut		Gland		Total	
						Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
					P falciparum						
1	150	927	Kamaladasi	4-2-31	14-2-31	2	0	2	0	2	0
2	153	1049	Habib	7-2-31	18-2-31	6	0	6	0	6	0
3	184	2736	Dhananjai	11-4-31	22-4-31	1	0	1	0	1	0
4	185	2960	Banku	15-4-31	27-4-31	2	0	2	0	2	0
					Total	11	0	11	0	11	0
					P vivax						
1	155	1137	Sufal	11-2-31	25-2-31	3	0	3	0	3	0
2	157	1209	Jaboona	11-2-31	23-2-31	1	0	1	0	1	0
3	164	1789	Latifan	7-3-31	16-3-31	2	1	2	0	2	1
					Total	6	1	6	0	6	1
					P malariæ						
1	171	2061	Mofussudin	18-3-31	27-3-31	1	0	1	0	1	0

The records detailed above suggest that *A hyrcanus* is not susceptible to infection with *P falciparum* and that it is susceptible to *P vivax* infection One specimen out of six fed on *P vivax* gametocyte carriers was found infected The infected specimen had a single oocyst in a nearly full-grown stage in which sporozoites could be recognized The negative result in one observation with *P malariæ* is not conclusive

(*f*) *Anopheles jamesi* Theobald

A single infection experiment was carried with *A jamesi* bred out of larvæ collected from Dam Dim (Jalpaiguri District, Bengal)

P falciparum

Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
					Gut		Gland		Total	
					Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
118	10064	Kalicharan	29-10-30	12-11-30	4	0	4	2	4	2

Sporozoites were seen in the salivary glands of two out of four specimens examined This experiment shows that *A jamesi* is capable of transmitting subtertian infection experimentally

(*g*) *Anopheles ludlowi* var *sundaica* Roden

The specimens used in these experiments were bred out of larvæ collected at Budge-Budge (24-Parganas District, Bengal)

Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
					Gut		Gland		Total	
					Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
				P falciparum *						
122	11210	Tarakdasi	19-11-30	4-12-30	1	1	1	0	1	1
				P vivax *						
119	10785	Usha	12-11-30	22-11-30	1	0	1	0	1	0
				P malariæ *						
120	11036	Haru	15-11-30	4-12-30	6	0	6	1	6	1

* The three results cited here were mentioned previously in another connection (Iyengar, 1931)

The results show that *A ludlowi* could be infected experimentally with *P falciparum* and *P malariæ* In the case of the latter infection sporozoites were observed in the salivary glands The negative result with *P vivax* is not of much value as only a single observation was made

(*h*) *Anopheles maculatus* Theobald

The number of observations made with this species and with *A majidi* is too small to be of value

P falciparum

Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
					Gut		Gland		Total	
					Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
118	10064	Kalicharan	29-10-30	12-11-30	1	0	1	0	1	0

(*i*) *Anopheles majidi* Young and Majid

P vivax

Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
					Gut		Gland		Total	
					Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
125	11767	Lakshmi	3 12-30	12-12-30	1	0	1	0	1	0

(*j*) *Anopheles minimus* Theobald

Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
					Gut		Gland		Total	
					Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
				P falciparum						
118	10064	Kalicharan	29-10-30	12-11-30	1	1	1	1	1	1
				P vivax						
125	11767	Lakshmi	3-12-30	12-12-30	10	1	10	1	10	1

The specimens used in these experiments were bred out of larvæ collected at Dam Dim (Jalpaiguri District, Bengal) *Anopheles minimus* was experimentally infected with tertian and subtertian parasites in the above recorded experiments

(*k*) *Anopheles pseudojamesi* Str and Ch

P malariæ

Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
					Gut		Gland		Total	
					Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
233	7906	Subode	19-8-31	31-8-31	1	0	1	0	1	0

(*l*) *Anopheles stephensi* Liston

The number of observations with this species is fairly large

P falciparum

Serial number	Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
						Gut		Gland		Total	
						Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
1	50	4496	Anil	26-7-30	8-8-30	16	2	16	0	16	2
2	65	5209	Tarak	6-8-30	20-8-30	11	3	11	5	11	5
3	71	5828	Tarak	16-8-30	22-8-30	3	2	3	0	3	2
4	83	6665	Dasu	3-9-30	10-9-30	4	1	4	0	4	1
5	121	11210	Tarakdasi	19-11-30	4-12-30	6	6	6	6	6	6
6	130	12367	Kendu	17-12-30	3-1-31	11	8	11	4	11	8
7	135	12727	Dasarathi	24-12-30	8-1-31	2	2	2	2	2	2
8	148	782	Muchiram	31-1-31	11-2-31	7	4	7	4	7	6
9	154	1075	Achiram	7-2-31	19-2-31	15	15	15	7	15	15
10	172	2056	Kamala	18-3-31	27-3-31	2	1	2	0	2	1
11	184	2736	Dhananjai	11-4-31	22-4-31	4	3	4	0	4	3
12	185	2960	Banku	15-4-31	27-4-31	4	3	4	0	4	3
13	189	3193	Sambu	25-4-31	5-5-31	3	1	3	0	3	1
14	196	4022	Guiram	16-4-31	30-5-31	5	1	5	0	5	1
15	199	4022	Guiram	20-5-31	30-5-31	3	3	3	2	3	3
16	202	4078	Gaur	23-5-31	5-6-31	15	15	15	1	15	15
17	208	4443	Abdul Malik	27-5-31	9-6-31	8	1	8	0	8	1
18	212	5070	Sisubala	20-6-31	3-7-31	3	0	3	1	3	1
					Total	122	71	122	32	122	76

It will be observed that positive results were obtained in every one of the batches of *A stephensi* fed on infective gametocyte carriers of *P falciparum* Out

of 122 mosquitoes examined, 71 were positive for oocysts, 32 for sporozoites in the salivary glands and 76 had either sporozoites or oocysts or both The minimum time taken for *sporozoites* to be found in the salivary glands has been found to be 11 to 12 days and in one instance as shown by the results in experiment No 199, only 10 days The results show that *A stephensi* is very susceptible to experimental infection with *P falciparum*

P vivax

Fifteen infection experiments were carried out with infective *P vivax* gametocyte carriers

Serial number	Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
						Gut		Gland		Total	
						Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
1	72	5961	Anath	20-8-30	30-8-30	9	1	9	0	9	1
2	123	11235	Jibau	19-11-30	5-12-30	10	4	10	5	10	6
3	124	11603	Guiram	29-11-30	8-12-30	11	8	11	2	11	8
4	128	12197	Kartic	13-12-30	26-12-30	15	9	15	12	15	13
5	131	12594	Haru	20-12-30	6-1-31	8	1	8	5	8	6
6	133	12510	Sudabala	20-12-30	8-1-31	1	0	1	0	1	0
7	143	426	Sarala	17-1-31	27-1-31	5	4	5	1	5	4
8	144	450	Panchi	21-1-31	2-2-31	13	5	13	10	13	11
9	145	598	Kristo	24-1-31	3-2-31	7	2	7	0	7	2
10	155	1137	Sufal	1-2-31	25-2-31	6	0	6	3	6	3
11	157	1209	Jaboona	11-2-31	23-2-31	8	3	8	0	8	3
12	195	3965	Jitendra	13-5-31	25-5-31	1	1	1	1	1	1
13	211	5004	Dulal	17-6-31	29-6-31	6	1	6	3	6	3
14	213	5090	Durgabala	20-6-31	3-7-31	11	3	11	6	11	7
15	226	6255	Lakshmi	22-7-31	10-8-31	1	1	1	0	1	1
					Total	112	43	112	48	112	69

The results show that *A stephensi* is very susceptible to *P vivax* infection when fed on infective gametocyte carriers In the present series of observations a successful infection of *A stephensi* was observed in fourteen out of fifteen infection experiments Out of a total number of 112 mosquitoes dissected after the infective feed, 69 showed an infection either with oocysts or sporozoites or both Sporozoites were observed in the salivary glands in 48 out of 112 specimens examined

P malariæ

Serial number	Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
						Gut		Gland		Total	
						Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
1	79	6380	Amar	27-8-30	10-9-30	8	1	8	1	8	1
2	120	11036	Haru	15-11-30	4-12-30	14	2	14	7	14	7
3	169	1954	Gokul	14-3-31	24-3-31	1	1	1	1	1	1
4	171	2061	Mofazudin	18-3-31	27-3-31	5	3	5	0	5	3
5	173	2349	Sudhir	25-3-31	4-4-31	11	1	11	0	11	1
6	218	5496	Banamali	1-7-31	16-7-31	14	3	14	0	14	3
7	233	7906	Subode	19-8-31	31-8-31	1	0	1	0	1	0
					Total	54	11	54	9	54	16

Positive results were obtained in six out of seven experiments Out of a total of 54 observations, 16 specimens took the infection and in nine specimens sporozoites were observed in the salivary glands As compared with its susceptibility to the two other species of *Plasmodium*, *A stephensi* appears to be less susceptible to infection with *P malariæ* than with *P vivax* or *P falciparum*

(*m*) *Anopheles varuna* Iyengar

Specimens of *A varuna* were fed on infective gametocyte carriers of all the three species of *Plasmodium*

P falciparum

Serial number	Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
						Gut		Gland		Total	
						Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
1	122	11210	Tarakdasi	19-11-30	4-12-30	1	0	1	0	1	0
2	133	12510	Sudabala	20-12-30	8-1-31	4	1	4	0	4	1
3	134	12705	Basu	24-12-30	8-1-31	4	0	4	1	4	1
4	147	782	Muchuram	31-1-31	11-2-31	3	2	3	1	3	2
5	153	1049	Habib	7-2-31	18-2-31	1	0	1	0	1	0
6	230	6742	Nalini	1-8-31	10-8-31	1	0	1	0	1	0
					Total	14	3	14	2	14	4

Out of 14 observations, infection was observed in 4, sporozoite infections of the salivary glands were observed in two specimens

P vivax.

Serial number	Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
						Gut		Gland		Total	
						Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
1	127	12197	Kartik	13-12-30	26-12-30	7	2	7	0	7	2
2	139	19	Upendra	7-1-31	19-1-31	3	0	3	0	3	0
3	144	450	Panchi	21-1-31	3-2-31	1	1	1	1	1	1
4	145	598	Kristo	24-1-31	3-2-31	5	0	5	0	5	0
					Total	16	3	16	1	16	3

Three out of 16 specimens of this species took infection with *P vivax* and sporozoites were observed in the salivary glands of one specimen

P malariæ

Experiment number	Ticket number	Name of gametocyte carrier	Date of feed	Date of dissection	RESULTS					
					Gut		Gland		Total	
					Number examined	Number positive	Number examined	Number positive	Number examined	Number positive
233	7906	Subhode	19-8-31	31-8-31	1	1	1	0	1	1

In this experiment one quartan oocyst was observed in a poorly developed condition on the 12th day after feed The above-mentioned experiments show that *A varuna* is susceptible to experimental infection with *P vivax* and *P falciparum* In both infections the development of the parasites proceeded to the stage of sporozoite infection of the salivary glands As regards its susceptibility to *P malariæ*, the number of experiments carried out in this series is too small to be of much value

Relative susceptibility of species of Anopheles to infection

In view of the fact that negative results do not form conclusive evidence, especially where the number of observations is small, only positive findings have been taken into consideration in the following discussion of the results —

P falciparum

Among the several species of *Anopheles* on which experimental infection was attempted, seven species were observed to be susceptible to experimental infection with *P falciparum*, namely, *A aconitus, fuliginosus, jamesi, ludlowi, minimus, stephensi* and *varuna* In all of them, except in *A ludlowi*, the infection developed to the stage of sporozoites in the salivary glands Only a single observation was made with *A ludlowi*, and in this case a large number of oocysts (more than 20) were seen in the gut , many of the oocysts were mature and contained well-developed sporozoites

A summary of the results of positive findings of infection with *P falciparum* is furnished below —

P falciparum

Species of Anopheles	Gut		Salivary glands		Total	
	Number examined	Number positive for oocysts	Number examined	Number positive for sporozoites	Number examined	Number positive for oocysts or sporozoites or both
A aconitus	1	1	1	1	1	1
A fuliginosus	58	19	58	1	58	20
A jamesi	4	0	4	2	4	2
A ludlowi var *sundaica*	1	1	1	0	1	1
A minimus	1	1	1	1	1	1
A stephensi	122	71	122	32	122	76
A varuna	14	3	14	2	14	4

Of the several species found susceptible to *P falciparum* infection, *A fuliginosus* showed the least tendency to develop a sporozoite infection, as only 1 out of 20 positives had sporozoites in the salivary glands Heavy oocyst infections were often observed in this species and, in several instances the oocysts were mature and contained fully formed sporozoites But as compared with the other susceptible species, *A fuliginosus* appears to be less susceptible to infection and the time taken for oocysts to mature was observed to be longer than in the other species

P vivax

Six species of Anopheles were observed to be susceptible to infection with *P vivax*, namely, *A aconitus, fuliginosus, hyrcanus, minimus, stephensi* and *varuna* Sporozoite infection of the salivary glands was observed in all of them, except in *A hyrcanus* and *A minimus*

An oocyst infection was observed in one specimen of *A hyrcanus* out of six specimens dissected The positive specimen had a single large oocyst with fully formed sporozoites The records are furnished below —

P vivax

Species of Anopheles	Gut		Salivary glands		Total	
	Number examined	Number positive for oocysts	Number examined	Number positive for sporozoites	Number examined	Number positive for oocysts or sporozoites or both
A aconitus	1	1	1	1	1	1
A fuliginosus	40	4	40	6	40	9
A hyrcanus	6	1	6	0	6	1
A minimus	10	1	10	0	10	1
A stephensi	112	43	112	48	112	69
A varuna	16	3	16	1	16	3

P malariæ

Only four species were observed to be susceptible to infection with *P malariæ*, while all the others gave negative results The species observed to be susceptible to infection with *P malariæ* are *A culicifacies, ludlowi, stephensi* and *varuna* The results are furnished below —

P malariæ

Species of Anopheles	Gut		Salivary glands		Total	
	Number examined	Number positive for oocysts	Number examined	Number positive for sporozoites	Number examined	Number positive for oocysts or sporozoites or both
A culicifacies	2	1	2	0	2	1
A ludlowi var *sundaica*	6	0	6	1	6	0
A stephensi	54	11	54	9	54	16
A varuna	1	1	1	0	1	1

Of the four species, sporozoite infection of the salivary glands was observed in only two species, namely, *A ludlowi* and *A stephensi* * One specimen of *A ludlowi* var *sundaica* and nine specimens of *A stephensi* were observed to develop a sporozoite infection of the salivary glands These results are of considerable interest in view of the difficulty which workers have experienced in producing a sporozoite infection of salivary glands with quartan parasites

James (1931) has shown how very difficult it is to obtain a sporozoite infection of the salivary glands with quartan parasites As Boyd (1930) says, 'there are but few authentic records of experimental transmission of *P malariæ* by means of mosquitoes' Various workers using different species of *Anopheles* have experienced difficulty in obtaining a salivary infection of the mosquito with *P malariæ* and although several of them succeeded in getting oocyst infections successful sporozoite infections of the salivary glands have been very rare James (1931, p 482) summarizes the previous findings as follows —

'It does not appear from the papers I have read that Grassi ever succeeded in cultivating the quartan parasite in mosquitoes beyond the stage of small oocysts in the stomach wall and, so far as I can ascertain, no later Italian worker seems to have been more successful Jancso made eighteen feeding experiments on cases infected with this parasite, but only four were successful (zygotes in the stomach wall) and none of his three experiments to transmit the parasite from mosquito to man had a positive result He remarked, "It is very difficult to obtain a successful infection with quartan malaria" and his tabular statement of the period required at different temperatures for *maculipennis* to become infective (sporozoites in the glands) contains only one record relating to quartan infection Stephens and Christophers, in 1901, fed specimens of five species of Indian Anopheles repeatedly on four different cases, but nearly two-thirds of all the mosquitoes used failed to become infected, and two was the largest number of zygotes found in any mosquito Sporozoites in the glands were found in only one of the five species of mosquito used Swellengrebel in the Dutch East Indies fed four species on two patients whose gametocytes numbered from 12 to 23 per 500 leucocytes, but only two specimens out of 300 became infected Walker and Barber, in the Philippines in 1913, had no successful result in twenty-four experiments Recently Dr Richard Green in the Institute for Medical Research, Federated Malay States, has reported that, as regards sporozoites in the glands of *A maculatus* fed upon quartan cases, his results have invariably been negative, though in several experiments there were small zygotes in the stomach wall' Recently there have been two reports of

* It was mentioned (Iyengar, 1931, footnote on p 511) that the author had effectively produced a salivary gland infection in *A stephensi*, *A ludlowi* and *A fuliginosus* with quartan parasites The last mentioned species has been omitted here for the reason that the gametocyte carrier on whom this species was fed was subsequently found to have a mixed infection of quartan with tertian

experimental infection of Anopheles with quartan parasites Averbukh (1930) reported that he transmitted quartan infection to himself through the bites of *Anopheles maculipennis* var *sacharovi* fed experimentally on a patient suffering from quartan malaria He fed three of the mosquitoes on himself on the 36th day after the infective feed and he developed quartan malaria 32 days later Anazawa (1931) reports the finding of sporozoite infection of the salivary glands in three specimens of *A fuliginosus* fed experimentally on a quartan carrier[1]

Infectivity of the gametocyte carrier to the mosquito

Hitherto we did not take into consideration those experimental feeds which failed to produce an infection in the mosquito, and considered only those that proved infective to the mosquito after a single feed When one considers the number of experiments which resulted in a successful infection (oocysts or sporozoites) in relation to the total number of experiments, there are some outstanding differences in the relative infectivity of gametocyte carriers of the three species of *Plasmodium*

Species	Total number of gametocyte carriers on whom mosquitoes were fed experimentally	Number that proved to be infectious to the mosquito in a single feed	Effective infectivity rate among gametocyte carriers
P falciparum	37	25	67 6 per cent
P. vivax	45	18	40 0 „
P malariæ	33	7	21 2 „

[illegible] of 37 *P falciparum* gametocyte carriers, 67 6 per cent were observed to be [illegible] to the mosquito in a single feed In the case of *P vivax*, 40 per cent of the [illegible] observed to be capable of effecting a mosquito infection and the effective [illegible] lowest (21 2 per cent) among *P malariæ* gametocyte carriers As [illegible] pp 67-68) says 'Attempts to induce experimental infection succeed with *P falciparum*, somewhat less readily with *P vivax* and are difficult with *P malariæ*' The variations in the relative infectivity of

[1] (*Pub Health Repts*, U S Treasury Department, **47**, No 35, Aug 26, 1932, [illegible] negative results in infection experiments with *A quadrimaculatus* and *A crucians*, with *A punctipennis* fed on quartan carriers In one specimen of *A punctipennis* [illegible] sites in the salivary glands

gametocyte carriers in the three infections are probably largely responsible for this difference It was thought that the greater ease with which mosquitoes could be infected experimentally with *P falciparum* was due to the more intensive infections that occur in subtertian infections and that the difficulty of infecting the mosquito with *P malariæ* was due to sparseness of gametocytes in the blood This view that mosquito infection is dependent on the intensity of gametocytes in the blood does not hold good One frequently observes that heavy gametocyte carriers are poor infectors, while carriers with low gametocyte counts often infect mosquitoes effectively Green has shown how carriers with very low gametocyte counts can serve to effect a mosquito infection and that negative results are by no means rare with even heavily infected carriers James' summary of Green's results is quoted below —

Lowest number of gametocytes when some individual members of a batch of *A maculatus* became infected			Highest number of gametocytes when no member of a batch of *A maculatus* became infected		
P falciparum	*P vivax*	*P malariæ*	*P falciparum*	*P vivax*	*P malariæ*
1 gametocyte to 200 leucocytes or 42 per c mm of blood	1 gametocyte to 1 000 leucocytes or 10 per c mm of blood	1 gametocyte to 330 leucocytes or 27 per c mm of blood	60 to 100 leucocytes or 2,310 per c mm of blood	12 per 100 leucocytes or 900 per c mm of blood	6 per 100 leucocytes or 300 per c mm of blood

James (1931) experienced failures in infecting mosquitoes from carriers with gametocyte counts as high as or even higher than those with which Green obtained negative results

From the present series of observations a similar statement could be made of the results of infection experiments with *Anopheles stephensi* —

Lowest number of gametocytes when at least one mosquito developed the infection			Highest number of gametocytes when none of the mosquitoes developed an infection		
P falciparum	*P vivax*	*P malariæ*	*P falciparum*	*P vivax*	*P malariæ*
1 gametocyte to 100 leucocytes	1 gametocyte to 200 leucocytes	1 gametocyte to 200 leucocytes	20 gametocytes to 100 leucocytes	16 gametocytes to 100 leucocytes	8 gametocytes to 100 leucocytes

These results show that in each of the species of *Plasmodium* the richness of gametocytes in the blood is not the factor which determines the infection of the mosquito It has been possible to obtain an infection of the mosquito in a single feed with very poor gametocyte counts and to experience failures with carriers having a much higher gametocyte incidence

Another view put forward to explain the difficulty in obtaining quartan infections of mosquitoes is on the basis of the sex-ratio among gametocytes It is thought that, in infections in which male and female forms are present in nearly equal numbers, greater chances are offered for an infection of the mosquito than in those in which the male and female forms are disproportionate in number But there is little evidence to show that an equal distribution of male and female forms occurs in *P falciparum* infections and that it is less equal in the other two species

James (1931) has shown that the quality of the gametocytes is the main factor which determines mosquito infection and that intensity of infection is not of any great significance, negative results are not uncommon in experimental feeds on even very heavy gametocyte carriers in which the gametocytes had not reached the particular stage of 'ripeness' He judges the infectivity of the carrier by the presence of a sufficient number of quickly exflagellating micro-gametocytes But, as he says, this test is a good indication in *P vivax* gametocytes whereas with regard to *P malariæ* and *P falciparum* 'even the finding of considerable numbers of exflagellating male forms has not proved a sure sign that *maculipennis* will become infected'

The difference in the relative infectivity of the gametocytes of the tertian, subtertian and quartan parasites mentioned previously is largely due to difference in the quality or ripeness of the gametocytes, judged from these results the incidence of ripe gametocytes is highest among *P falciparum*, less frequent in *P vivax* and least in *P malariæ* Similarly gametocyte carriers that are infective to the mosquito form a comparatively high proportion in the case of *P falciparum* infections and a very low proportion in *P malariæ* infections

Effect of fruit juice on the development of oöcysts

In all these infection experiments laboratory-bred mosquitoes were fed on raisins until the day of the infective feed, and after the infective feed they were kept solely on raisins The experimental feed on the gametocyte carrier comprises the only blood feed that the mosquitoes got, and they were kept on raisins both before and after the single experimental feed The positive results in many of these experiments show that feeding mosquitoes on raisins has no inhibitory influence on the development of oocysts Heavy oocyst infections have often been observed in this series of experiments and in a large number of cases the oocysts developed

well and produced sporozoites in due course This confirms the findings of James (1931) that feeding mosquitoes on fruit is not deleterious to oocyst production and development

SUMMARY

Infection experiments were carried out with several species of *Anopheles* by feeding laboratory-bred mosquitoes on gametocyte carriers Seven species were found susceptible to experimental infection with *P falciparum*, namely, *A aconitus*, *A fuliginosus*, *A jamesi*, *A ludlowi* var *sundaica*, *A minimus*, *A stephensi* and *A varuna* Of these, *A fuliginosus* appeared to be less susceptible to infection than the other species

Six species of *Anopheles* were observed to be susceptible to infection with *P vivax*, namely, *A aconitus*, *A fuliginosus*, *A hyrcanus*, *A minimus*, *A stephensi* and *A varuna* Of these, *A hyrcanus* showed the least susceptibility to infection

Four species were observed to be susceptible to infection with *P malariæ*, namely, *A culicifacies*, *A ludlowi*, *A stephensi* and *A varuna* Sporozoite infections of the salivary glands were observed in two species *A ludlowi* and *A stephensi*

The proportion of gametocyte carriers that proved to be infective to *Anopheles* varied in the three *Plasmodium* infections The effective infectivity rates among gametocyte carriers were found to be *P falciparum* 67 6 per cent, *P vivax* 40 0 per cent and *P malariæ* 21 2 per cent The variation in the incidence of 'ripe' gametocytes in the three infections seems to be the reason for the difference in the effective infectivity of gametocyte carriers

Feeding mosquitoes on fruit has no inhibitory influence on the formation or development of oocysts

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THE VITAMIN B CONTENT OF DIFFERENT SAMPLES OF INDIAN RICE BY SPRUYT'S COLORIMETRIC METHOD [1]

Part I

BY

SUDHAMOY GHOSH, M SC, D SC, F R S E,

AND

ASHUTOSH DUTT, B SC

(*From the School of Tropical Medicine and Hygiene, Calcutta*)

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A COLORIMETRIC method for the estimation of vitamin A, based upon its reaction with arsenic trichloride or antimony trichloride, has long been known and much work has been carried out on the subject A chemical method for the estimation of other vitamins has been wanting and only very recently Spruyt (1930) has worked out a colorimetric method for the estimation of vitamin B in samples of rice By feeding experiments on rice birds (*Munia maja* and *Munia leucogastroides*) he has shown that the results of his colorimetric method run parallel to the clinical results, a fact which appears to us to afford a better future for this method than the one for vitamin A

We had occasion to isolate some vitamin B from rice polishings by Jansen and Donath's method (1927), in order to study its pharmacological action During the course of investigations on the beri-beri problem of Bengal by Lieut -Colonels Acton and Chopra it seemed desirable to find out the relative vitamin B contents of some common Indian samples of rice We tested over 50 samples of rice collected in or near Calcutta either from the rice dealers or from some of the rice mills A few samples were also obtained direct from Burma The investigation has elicited a few interesting points and we are recording these in this first report

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[1] An abstract of this paper was sent to the Indian Science Congress in January 1931, but the detailed paper was not published earlier as we were waiting to confirm some of our results by simultaneous feeding experiments and colorimetric estimations These have now been carried out and the results will be communicated in another paper

Spruyt's method is described as follows —

'Ten grammes of rice (whole grains), 50 c c of Solution A (a 1 per thousand solution of salicylic acid to which were added a quarter per cent solution of sulphuric acid and a few drops of toluol), 5 grammes of Norite (a proprietary charcoal) and 5 drops of toluol were taken in a wide mouthed bottle of 300 c c capacity and slowly rocked (± 50 strokes per minute) for 20 hours The mass was then filtered and 20 c c of the filtrate were pipetted into a centrifuge tube and 4 c c of a solution of phospho tungstic acid (Solution B, containing 50 grammes of dry phospho tungstic acid per 100 c c of solution) were added The solution was then cooled in a freezing mixture of ice and salt for a few hours and then placed in a thermostat at 30°C for 12 hours, it was next centrifuged in a Runne centrifuge (± 3,000 revolutions per minute) after adding half gramme of very finely divided filter paper The clear liquid was decanted, the precipitate was stirred twice with 20 c c of Solution A and centrifuged The precipitate was then transferred with HCl (sp gr ± 1 1) to a 500 c c wide mouthed flask, reduced with zinc until the final brownish red colour was attained The contents of the flask were transferred to a 250 c c measuring flask and a little stannous chloride was added to make the colour more permanent After cooling, the flask was filled up to the mark, the liquid was filtered off and examined colorimetrically by comparison with a standard colour gauged upon a glass slide The colour of the slide appeared to correspond to a column of 38 mm of a solution obtained by the reduction of 10 c c of a solution of phospho tungstic acid (2 071 grammes of dry substance per 100 c c) diluted up to 250 c c Besides this, a blank test (20 c c Solution A and 4 c c Solution B) was carried out in each series to determine the colour due to the phospho tungstic acid retained by the filter-paper The indices obtained proved to be fairly constant with variations of at most 10 per cent'

We made some slight modifications in the method to suit our laboratory conditions and we describe in detail some of the points which we found helpful in order to secure more uniform results —

Ten grammes of rice (whole grains), 5 grammes of Norite (obtained from Java), 50 c c of Solution A and a few drops of toluene were taken in a 250 c c bottle which was then tightly closed with a rubber-stopper, made secure and then placed in a shaking apparatus The strokes were of moderate intensity and the shaking was continued overnight until 20 hours were completed (We also found by trial that by reducing the time of shaking to 10 hours, the colour-index obtained was the same) The extract was filtered through a dry filter and 20 c c of the filtrate were taken in a centrifuge tube of about 28 c c capacity (diameter 1 inch, length 3½ inches) Four c c of Solution B were then added, the solution gently mixed and left overnight in a cool place to settle The precipitate which adhered to the sides of the tube was scraped down with a glass-rod and mixed with 0 3 gramme of dry paper-pulp (prepared beforehand from good quality of filter-paper and dried) It was then centrifuged for 5 minutes at 3,000 revolutions per minute The supernatant liquid was pipetted off and the precipitate washed twice with 20 c c of Solution A, *centrifuging each time for 5 minutes** The precipitate mixed with the paper-pulp was then carefully transferred with 25 c c of HCl (sp gr 1 1) to a conical flask of about 250 c c capacity As no coloured glasses were

* In the absence of a large centrifuge, we found that the work could be carried out with equal satisfaction by dividing the solution in two smaller centrifuge tubes and using revolutions of not more than 2,000 per minute, the time of centrifuging being increased to 10 minutes

available for comparison, we used another similar flask, in which was placed 0 3 gramme of paper-pulp, 10 c c of a standard solution (containing 2 071 per cent of pure, dry phospho-tungstic acid) and 25 c c of HCl (sp gr 1 1)

The solutions were then reduced with pure, arsenic-free granulated zinc, the acid and zinc being replaced as soon as the reaction appeared to slow down The flasks were constantly shaken to help a uniform and quick reduction and they were provided with Bunsen valves to avoid re-oxidation About 80 c c of the HCl were necessary to complete the reduction The process of reduction occupied about 2 hours and as daylight proved more suitable for the readings with the colorimeter, we found it more expedient to start the reduction early in the day An economy of time is effected by starting 6 or more samples at a time and 18 samples can be easily managed per week

During the reduction the solution first turns blue, which gradually changes to blue-black and finally to brown When the final change had taken place, a pinch of solid stannous chloride was added and it was dissolved by shaking The solution was then filtered into a 250 c c measuring cylinder, the paper-pulp washed carefully with another 20 c c of the HCl to avoid precipitation and the solution finally made up to 250 c c with distilled water

The colour of the solution was matched with the standard in an Leitz Wetzlar Colorimeter keeping the height of the standard at 38 mm The *colour-index* number was then calculated, taking that of the standard as 100 As the solutions gradually deposit a precipitate with a change of shade, all the comparisons should be finished as quickly as possible

A few blank tests with 20 c c of Solution A and 1 c c of Solution B were carried out to determine the colour which could be imputed to any phospho-tungstic acid retained by the filter-paper , the results were all negative By starting the reduction of the standard at the same time and under exactly similar conditions as the unknown, any slight error due to the stage of reduction was avoided In order to test whether we were getting uniform results, we repeated the examination of some of the samples 5 or 6 times

TABLE

Serial numbers	Classification and description of rice	Source	Colour index	REMARKS
	Burma Rice (sun dried)			
1	'Rangoon Atap,' first quality	Local dealer	107	
2	'Emata Atap'	Prome	122	
3	'Ngasein Atap'	Pwinbyu	117	
4	'Kamakyi Atap'	Hanthawaddy	128	
5	'Letywezin Atap'	Insein	126	

of the selected red grains was found to be practically the same as that of the whole sample, viz , 161 The same rice, when polished, showed a colour-index of only 122 A middle grade ' balam ' unpolished rice gave a colour-index of 196 (an average of 7 assays) and the same rice when polished had a colour-index of 166 The outer colour of rice is not, therefore, an indication of its increased vitamin B content and a good deal depends upon the degree of polishing The average of the 24 samples of ' atap ' rice is 110 and the average of the 22 samples of parboiled rice is 133 The average of the few samples of hand-hulled (dhenki') ' atap ' is only 84 and the average of the dhenki-hulled ' parboiled rice is 117 ' Atap ' rice, as found in the market, is generally thrice-hulled and thus shows a lower colour-index, and possibly a lower content of vitamin B, than parboiled rice, and this appears to be contrary to the prevalent belief Again, the samples of ' dhenki-hulled ' rice examined have been found to possess a lower colour-index than even ' mill-hulled ' rice, and this also appears to be contrary to the prevalent view in India The most important factor affecting the vitamin B content of rice appears to us to be the degree of polishing to which the grains have been subjected

Summary and conclusions —Over 50 samples of Indian rice were tested by Spruyt's colorimetric method recently published Some slight modifications of the original method have been made to suit the laboratory conditions and they are described in detail The results show that the colour of the grain is not always an indication of its higher colour-index and possibly its vitamin B content The average colour-index of the samples of ' atap ' rice, which is generally thrice-hulled, is found to be lower than the average for the parboiled samples The few samples of ' dhenki-hulled ' rice examined were also found to have a lower colour-index than the ' mill-hulled ' samples ' Bazar atap ' rice, which is generally thrice-hulled, may thus often have a lower content of vitamin B than parboiled rice, and ' dhenki-hulled ' rice may also often have a lower content of vitamin B than the ' mill-hulled ' sample, contrary to the usual belief in India The most important factor affecting the vitamin B content of rice appears to us to be the degree of polishing to which the grains have been subjected

In conclusion, we desire to express our grateful thanks to Lieut -Colonel H W Acton, C I E , I M S , who has all along taken a keen interest in the work and at whose suggestion we took up the investigation

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NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

Part XXXII

PHLEBOTOMUS DENTATUS N SP

BY

LIEUT-COLONEL J A SINTON, M D, D SC, I M S

(*From the Malaria Survey of India, Kasauli*)

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IN 1922 a collection of *Phlebotomus* preserved in spirit was received from the late Dr N Annandale, F R S, labelled as collected in 1911 by Lieut-Colonel Wimberley, I M S, in Quetta, Baluchistan These specimens after mounting in balsam were identified as *P minutus* var and as *P sergenti* Recently, on account of the diagnostic importance of the buccal and pharyngeal structures in this genus, it was decided to remount the former specimens to display these parts When this was done it was found that the specimens belonged to a new species Unfortunately, as is frequently the case with specimens preserved in spirit, many of the appendages were missing There were 2 females and 1 male in the collection and it is proposed that the new species be named *Phlebotomus dentatus*

Phlebotomus dentatus N SP (♀)

The measurements of the different parts of the two co-type females are given in Table I

The *total length* of the insect is about 2 4 mm and the hairs seem all to be recumbent on the dorsal surfaces of abdominal segments II—VI

The *buccal cavity* (Plate XXXVII, fig 4) has a curved armature consisting of four very large pointed teeth on each side and six much smaller median ones No trace of a pigmented area could be seen

The *pharynx* (Plate XXXVII, fig 3) has a well-developed armature consisting of numerous long slender teeth anteriorly, with smaller teeth posteriorly and towards the middle line The greatest width of the pharynx is about twice its narrower anterior portion and its length is about $2\frac{1}{2}$ times its greatest breadth

The *antennae* are absent in both specimens

The *palps* (Plate XXXVII, fig 2) were very distorted in one specimen but the formula seems to be 1, 2, 4, 3, 5 Newstead's spines are situated on the

basal half of the 3rd segment and number about 40 This segment is much stouter than segment 4

TABLE I

Phlebotomus dentatus N SP (♀ ♀)

	Structures	Lengths in mm of specimens number — 1	2	Remarks, relative lengths, etc
BODY	Clypeus and head	0 343	0 343	
	Thorax	0 714	0 643	
	Abdomen	1 283	1 483	
	Total length	2 35	2 47	
MOUTH	Labium	0 230	0 214	
	Pharynx, length	0 150	0 156	= 2 44—2 5 × breadth
	Pharynx, breadth	0 060	0 063	
PALP	Segment 1	0 036	0 036	Formula 1, 2, 4, 3, 5
	Segment 2	0 081	0 078	
	Segment 3	0 117	0 132	
	Segment 4	0 096	0 108	
	Segment 5	0 170	0 201	
	Total length	0 500	0 555	
WING	Length	1 643	1 710	5 4 × breadth
	Breadth	0 300	0 314	$\frac{a}{\beta}$ = 0 55—0 60 $\frac{\beta}{\gamma}$ = 0 91—1 0
	a	0 157	0 170	$\frac{a}{\gamma}$ = 0 55—0 57 $\frac{\delta}{a}$ 0 27—0 41
	β	0 285	0 285	
	γ	0 285	0 314	$\frac{a}{\varepsilon}$ = 0 53 $\frac{\theta}{\varepsilon}$ = 2 6
	δ	0 043	0 070	
	ε	0 300	0 314	$\frac{a+\beta}{\theta}$ = 0 54—0 56 $\frac{\text{Wing}}{\theta}$ = 2 11—2 03
	θ	0 785	0 840 ?	

The *wings* (Plate XXXVII, fig 1) are poorly preserved in both specimens, but the measurements in Table I are approximately correct The wing is very lanceolate and nearly $5\frac{1}{2}$ times as long as broad β is about equal to γ, while a is a little more than half the length of either of these, the ratio δ over is about 0 3

The *spermathecae* (Plate XXXVII, figs 5 and 6) are large and pipe-shaped, with wide ducts resembling those of *P minutus* s str The furca has a fringed base The genital area shows several transverse rows of teeth

Differential diagnosis

The shape of the unsegmented spermatheca and the absence of erect abdominal hairs separates this species from all those of the erect-haired group The morphology of the buccal armature and the large size of the teeth differentiate it from the other members of the recumbent-haired division

Phlebotomus dentatus N SP (♂)

The measurements of the different parts of the type male are given in Table II

TABLE II

Phlebotomus dentatus N SP (♂)

	Structures	Length in mm	Remarks, relative lengths, etc
BODY	Clypeus and head	0 357	
	Thorax	0 500	
	Abdomen, proper	1 070	
	Superior clasper, seg 1	0 276	
	Total length	2 20	
MOUTH	Labium	0 170	
	Pharynx, length	0 120	= 3 33 × breadth
	Pharynx breadth	0 036	
PALP	Segment 1	0 030	Formula 1, 2 4, 3, 5
	Segment 2	0 075	Relative lengths 2 7, 6 8, 11 0, 10 0, 19 1
	Segment 3	0 120	
	Segment 4	0 110	
	Segment 5	0 210	
	Total length	0 546	
GENITALIA	Superior clasper, seg 1	0 276	= 2 19 × seg 2, 1 70 × inferior clasper
	Superior clasper, seg 2	0 126	= length of spines
	Intermediate appendage	0 225	= 0 81 × seg 1
	Intromittent organ	0 174	= 0 77 × intermediate appendage
	Genital filament	0 018	(Length protruded)
	Inferior clasper	0 258	= 1 32 × subgenital lamella
	Subgenital lamella	0 195	

The *total length* of the insect is about 2 2 mm

The *buccal cavity* (Plate XXXVII, fig 7) shows a number of large teeth on one side, those on the other cannot be seen No trace of a pigmented area was found

The *pharynx* (Plate XXXVII, fig 8) has several rows of stout teeth, about 6 in each anterior row and about 3 in the posterior ones The greatest width is about twice the narrow anterior portion and its length is almost $3\frac{1}{2}$ times its greatest breadth

The *antennae* are both missing

The *palps* (Plate XXXVII, fig 9) have a formula of 1, 2, 4, 3, 5 and the relative lengths of the segments are 2 7, 6 8, 11 0, 10 0 and 19 1 respectively Newstead's spines number about 7 or 8 and are situated on the basal half of the 3rd segment

The *wings* are both missing

The *male genitalia* (Plate XXXVII fig 11) are of the *minutus* type The distal segment of the superior claspei bears 4 long curved spines almost as long as the segment These spines arise apically Both this segment and the proximal one are long and narrow The intromittent organ (Plate XXXVII, fig 10) has a bluntly pointed end from which the genital filament is protruded for a short distance This portion of the filament is very curved and has a rounded end The ends of the intermediate appendages are not so markedly beak-shaped as in some other species

Differential diagnosis

The shape of the genitalia separate it from all the species with numerous erect hairs on the dorsum of the abdomen The absence of scars of erect hairs on the dorsal surfaces of abdominal segments II—VI differentiate the species from any of the sub-genus *Sintonius* The buccal armature and the long and narrow distal segment of the superior claspei seem characteristic of this insect

The genitalia of the male of *P dentatus* resemble those of *P fallax* Parrot (1921) described from North Africa There is the same long distal segment to the superior clasper and the same palpal formula Parrot (1921) stated that the 5th or non-deciduous spine was absent from the superior clasper but Nitzulescu (1931) and Theodor (1931) report that this spine is present but very atrophied and situated close to the other spines The spine figured by Nitzulescu (1931) is much smaller and more distal than that of *P dentatus* The narrower intromittent organ and the absence of a pigmented area also distinguish *P dentatus* from *P fallax*

The female of *P fallax* has not been described, so in the meantime *P dentatus* is considered to be a new species

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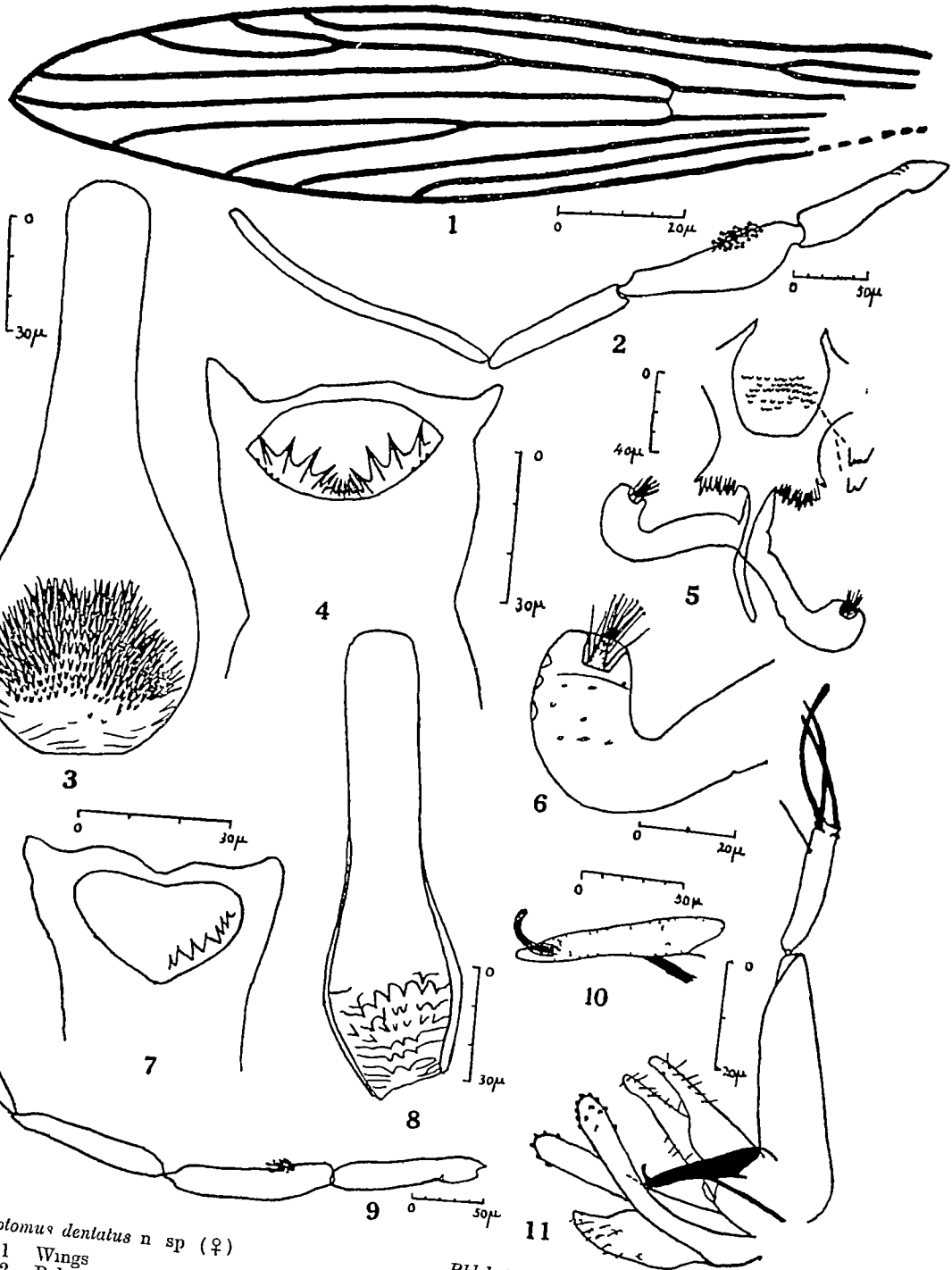

Phlebotomus dentatus n sp (♀)

Fig 1 Wings
" 2 Palps
" 3 Pharynx
" 4 Buccal cavity
Figs 5 and 6 Spermathecae

Phlebotomus dentatus n sp (♂)

Fig 7 Buccal cavity
" 8 Pharynx
" 9 Palps
" 10 Intromittent organ
" 11 Genitalia

Ind Jour Med Res, XX, 3, *January*, 1933

NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

Part XXXIII

PHLEBOTOMUS HODGSONI N SP

BY

LIEUT-COLONEL J A SINTON, M D, D SC, I M S

(*From the Malaria Survey of India, Kasauli*)

[Received for publication, September 13, 1932]

AMONG a collection of *Phlebotomus* received in 1923 from Major D Clyde, I M S, from Waziristan, was one labelled from 'M O's Tent, Jandola, 5th August, 1923' This specimen, which was a female, was unfortunately incomplete, but it appeared to be a new species belonging to the group with scanty erect abdominal hairs (sub-genus *Sintonius* of Nitzulescu, 1931) Since that time no more specimens of this species were received until this year Through the kindness of Colonel E C Hodgson, D S O, K H P, I M S, A D M S, Peshawar District, I have obtained several collections of *Phlebotomus* from the Peshawar area and among these were a number of specimens of this new species, for which I propose the name *Phlebotomus hodgsoni* My sincere thanks are due to Colonel Hodgson and also to Major C F Anthonisz, R A M C, and Captain A Sachs, R A M C, who made the collections

Specimens from the following places are now available for study —

(*a*) Jandola, Waziristan (about 2 000 feet)—5-8-23 (Clyde) 1 ♀

(*b*) Cherat, Peshawar District (4,500 feet)—(*i*) 14-6-32 (Sachs) 1 ♀, 21-6-32 (Sachs) 5 ♀ ♀, 2 ♂ ♂, 26-6-32 (Sachs) 2 ♀ ♀, 1 ♂ (*ii*) — -6-32, Wash-houses, Regimental Barracks (Anthonisz) 2 ♀ ♀, 3 ♂ ♂, Officers' Quarters, Haly's Hill (Anthonisz) 1 ♂ (*iii*) 1-7-32, Residential Quarters (Anthonisz) 1 ♂ (*iv*) 2-7-32, Wash-houses, Regimental Barracks (Anthonisz) 3 ♀ ♀, 1 ♂

(*c*) Landi Kotal, Peshawar District (3,500 feet)—24-6-32 (Sachs) 3 ♀ ♀, 2 ♂ ♂

Phlebotomus hodgsoni N SP (♀)

Appearances in dry state

General appearance a medium-sized, dark grey sandfly The integument of body dark brown, except sides of thorax lighter Wings with dark grey hairs and bluish-golden iridescence of membrane Abdominal hairs light brown, with few erect ones on dorsal surfaces of abdominal segments Integument of legs dark brown with silvery scales Halteres black

Appearances in stained and mounted specimens.

The measurements of the different parts of four females are given in Table I

TABLE I

Phlebotomus hodgsoni N SP (♀)

	Structures	Lengths in mm of specimens number — 1	2	3	4	Remarks, relative lengths, etc
BODY	Head and clypeus	0 370	0 400	0 400	0 400	
	Thorax	0 614	0 730	0 730	0 685	
	Abdomen proper	1 114	1 857	1 530	1 570	
	Superior clasper	0 170	0 143	0 170	0 170	
	Total length	2 27	3 1	2 8	2 8	
MOUTH	Labium	0 230	9 243	0 214	0 230	
	Pharynx, length	0 156	0 180	0 162	0 162	=3 1×breadth
	Pharynx, breadth	0 051	0 060	0 052	0 054	=1 9×narrowest part
ANTENNA	Segment III	0 120	0 147	0 147		<IV+V
	Segment IV	0 066	0 084	0 078		
	Segment V	0 069	0 084	0 080		Formula $= \frac{2}{\text{III—XV}}$
	Segment VI	0 066	0 087	0 084		
	Segments XII–XVI	0 250	0 315	0 306		
	Total length	1 030	1 260	1 230		=4 2×XII-XVI, 8 5×III
PALP	Segment 1	0 033	0 039	0 040	0 036	Formula 1, 2, 4, 3, 5
	Segment 2	0 072	0 084	0 080	0 081	Relative lengths 3 1, 6 5 13 5, 10 0 22 7
	Segment 3	0 150	0 174	0 159	0 168	=2 ×2nd
	Segment 4	0 108	0 130	0 120	0 126	
	Segment 5	0 264	0 276	0 270	0 285	Newstead's spines about 40
	Total length	0 627	0 703	0 669	0 696	

TABLE I—*concld*

	Structures	Lengths in mm of specimens number — 1	2	3	4	Remarks, relative lengths, etc
WING	Length	1 630	1 930	1 850	1 710	=4 2× breadth
	Breadth	0 385	0 457	0 430	0 400	$\frac{\alpha}{\beta}$ = 0 4 — 1 0 $\frac{\beta}{\gamma}$ =0 9 — 1 3
	α	0 135	0 270	0 285	0 200	
	β	0 350	0 330	0 285	0 314	$\frac{\alpha}{\gamma}$ = 0 5 — 0 9 $\frac{\delta}{\alpha}$ = 0 2 — 0 5
	γ	0 257	0 330	0 330	0 285	
	δ	0 030	0 093	0 143	0 071	$\frac{\alpha}{\varepsilon}$ = 0 3 — 0 6 $\frac{\theta}{\varepsilon}$ = 2 0 — 2 9
	ε	0 270	0 430	0 485	0 371	$\frac{\alpha+\beta}{\theta}$ = 0 6 $\frac{\text{Wing}}{\theta}$ = 2 1
	θ	0 785	0 914	0 930	0 843	
HIND LEG	Femur	0 670	0 814	0 770	0 757	
	Tibia	0 614	1 000	0 930	0 957	
	Tarsus, seg 1	0 343	0 514	0 485	0 430	
	Tarsus, segs 2–5	0 571	0 714	0 685	0 643	
	Total length	2 2	3 0	2 9	2 8	

The *total length* of insect about 2 3—3 0 mm Scars of few erect and many recumbent hairs seen on dorsal surfaces of abdominal segments II—VI

The *buccal cavity* (Plate XXXVIII, fig 3) has a large, well-developed pigmented area, an armature consisting of a single row of long contiguous teeth about 60 in number There is a marked triangular projection from each side of buccal cavity resembling those seen in *P squamipleuris* (Sinton, 1927) but without the marked serrations

The *pharynx* (Plate XXXVIII, fig 8) not much dilated posteriorly, length about 3 times greatest breadth, which is about twice the width of narrow anterior portion Armature of several rows of fine, comparatively short, teeth

The *antennae* (Plate XXXVIII, figs 5 and 6) with formula of 2 over III—XV, geniculate spines of basal segments stout and comparatively short, those of distal segments longer Segment III about half combined lengths of segments XII—XVI, but less than that of IV—V Total length about 4 times combined length of segments XII—XVI and more than 8 times segment III

The *palps* (Plate XXXVIII, fig 7) have formula of 1, 2, 4, 3, 5 , relative lengths of segments 3 1, 6 5, 13 5, 10 0, 22 7 Segments 1, 2 and base of 3 much stouter than rest of palp Newstead's spines number about 40 on basal third of segment 3

The *wing* (Plate XXXVIII, fig 4) lanceolate and distinctly pointed , about 4 2 times as long as broad Considerable variation in venation , one specimen had a negative δ

The *spermatheca* (Plate XXXVIII, fig 2) pipe-shaped, with body gradually merging into a wide short duct, uniting with one of opposite side (Plate XXXVIII, fig 1) The morphology of the spermatheca resembles that of *P minutus* Rond , but the organ shows transverse striation Some rows of minute spines in vulvar region

Differential diagnosis

The shape of the spermatheca differentiates *P hodgsoni* from the members of the recumbent-haired group The buccal cavity resembles in some degree that of *P squamipleuris* and *P hospitii*, but the pharynx is distinctly different and the spermatheca markedly so The morphology of the buccal cavity and the spermatheca differentiates it from the other members of the erect-haired group (*vide* Sinton, 1932)

Phlebotomus hodgsoni N SP (♂)

The measurements and ratios of four specimens are given in Table II

TABLE II

Phlebotomus hodgsoni N SP (♂)

	Structures	Lengths in mm of specimens number —				Remarks, relative lengths, etc
		1	2	3	4	
BODY	Head and clypeus	0 400	0 385	0 400	0 357	
	Thorax	0 640	0 643	0 670	0 530	
	Abdomen, proper	1 430	1 300	1 243	1 185	
	Sup claspei, seg 1	0 258	0 270	0 270	0 267	
	Total length	2 73	2 60	2 60	2 33	
PALP	Segment 1	0 033	0 036	0 039	0 033	Formula 1, 2, 4, 3, 5
	Segment 2	0 075	0 081	0 084	0 075	Relative lengths 3 0, 6 4, 12 6, 10 0, 22 0
	Segment 3	0 147	0 160	0 165	0 147	=2×2nd
	Segment 4	0 120	0 126	0 132	0 117	
	Segment 5	0 264	0 258	0 285	0 270	
	Total length	0 642	0 660	0 705	0 642	

TABLE II—*concld*

	Structures	Lengths in mm of specimens number — 1	2	3	4	Remarks, relative lengths, etc
ANTENNA	Segment III	0 144	0 144	0 150	0 144	<IV + V
	Segment IV	0 084	0 090	0 084	0 084	
	Segment V	0 085	0 090	0 090	0 087	Formula — $\frac{1}{\text{III—XV}}$
	Segment VI	0 085	0 091	0 090	0 087	
	Segments XII–XVI	0 291	0 324	0 285	0 315	
	Total length	1 250	1 270	1 200	1 240	=4 1×XII–XVI, 8 0–8 9×III
WING	Length	1 710	1 685	1 685	1 485	=4 2×breadth
	Breadth	0 410	0 400	0 400	0 350	
	α	0 200	0 200	0 170	0 150	$\frac{\alpha}{\beta}$ =0 50—0 65 $\frac{\beta}{\gamma}$ = 1 0—1 2
	β	0 307	0 301	0 300	0 300	
	γ	0 257	0 243	0 300	0 257	$\frac{\alpha}{\gamma}$ = 0 6—0 8 $\frac{\delta}{\alpha}$ = 0 2—0 3
	δ	0 057	0 071	0 043	0 030	
	ϵ	0 370	0 364	0 330	0 257	
	θ	0 857	0 830	0 800	0 700	$\frac{\alpha}{\epsilon}$ = 0 5—0 6 $\frac{\theta}{\epsilon}$ = 2 3—2 6
						$\frac{\alpha+\beta}{\theta}$ =0 60—0 65 $\frac{\text{Wing}}{\theta}$ = 2 0
HIND LEG	Femur	0 700	0 700		0 600	
	Tibia	0 900	0 914			
	Tarsus, seg 1	0 470	0 493			
	Tarsus, segs 2–5	0 630	0 628			
	Total length	2 7	2 7			(Not including coxa and trochanter)
GENITALIA	Sup clasper, seg 1	0 258	0 270	0 270	0 267	=2 2—2 3×seg 2, 1 1 × inferior clasper
	Sup clasper, seg 2	0 114	0 120	0 117	0 123	
	Intermediate append	0 216	0 216	0 219	0 216	=0 82×superior clasper, seg 1
	Intromittent organ	0 183	0 186	0 180	0 180	=0 84×intermediate appendage
	Genital filament	0 150	0 036	0 060	0 006	=Length protruded
	Inferior clasper	0 243	0 246	0 255	0 240	
	Subgenital lamellæ	0 201	0 204	0 216	0 192	=0 82×inferior clasper

Appearances in dry specimens

A dark grey, medium-sized sandfly, with dark greyish-brown integument, except sides of thorax which are light grey Thoracic and abdominal hairs brown A few erect hairs on dorsal surfaces of abdominal segments II—IV Wings with dark grey hairs and bluish-golden iridescence The integument of legs almost black and covered with silvery scales The antennæ and palps with dark grey hairs and lighter scales

Appearances in stained and mounted specimens

The measurements of the different parts of 4 males are given in Table II

The *total length* of the insect about 2 3—2 7 mm In some specimens scars of erect hairs very scanty or absent from dorsal surface of abdominal segments

The *buccal cavity* (Plate XXXIX, fig 12) has a distinct oval pigmented area The armature with about 50 small teeth

The *pharynx* (Plate XXXIX, fig 14) resembles that of female, but teeth more poorly developed

The *antennae* (Plate XXXIX, figs 10 and 11) with formula 1 over III—XV, geniculate spines smaller than in female Relative lengths very similar to those of female

The *palps* (Plate XXXIX, fig 9) with formula 1, 2, 4, 3, 5 and average relative lengths 3 0, 6 4, 12 6, 10 0, 22 0 Newstead's spines about 8—10 Basal segments not so stout as in female

The *male hypopygium* (Plate XXXIX, fig 15) of *minutus* type Distal segment of superior clasper with 4 curved apical or sub-apical spines, each almost as long as segment, non-deciduous spine arises about middle of segment Intermediate appendage (Plate XXXIX, fig 15) has a distinct ventral rudimentary lobe with about 8 short stout spines Intromittent organ tapers slightly to a blunt end, genital filament usually protruded Pompetta lies in abdominal segment 6 or 7

Differential diagnosis

The distinct, spinose, rudimentary lobe on the intermediate appendage, the morphology of the buccal cavity and the position of the non-deciduous hair, combined with its erect-haired character, differentiates this species clearly from all others having the *minutus* type of genitalia

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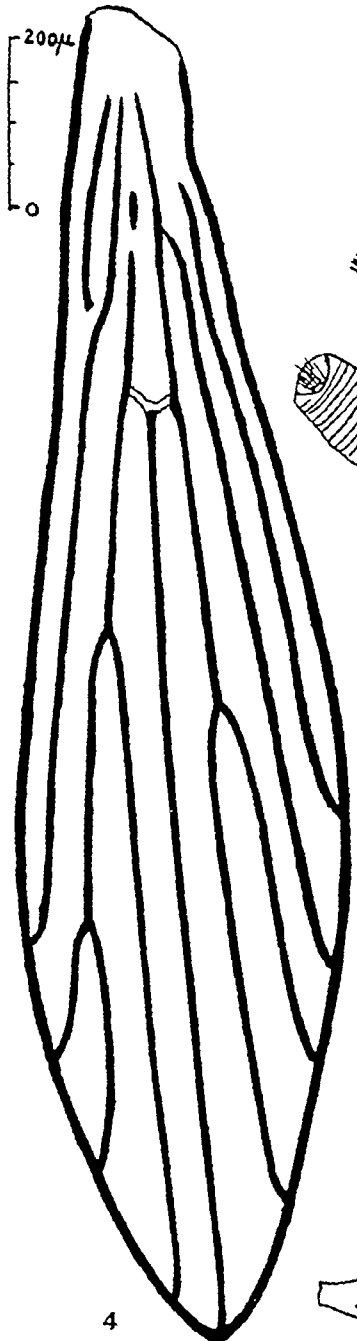
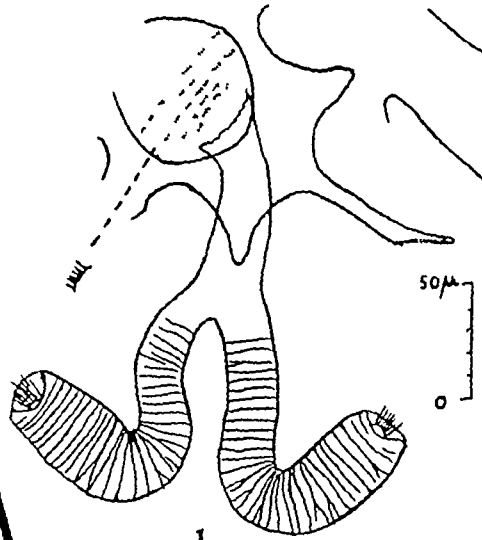
Plate XXXVIII

Phlebotomus hodgsoni n sp (♀)





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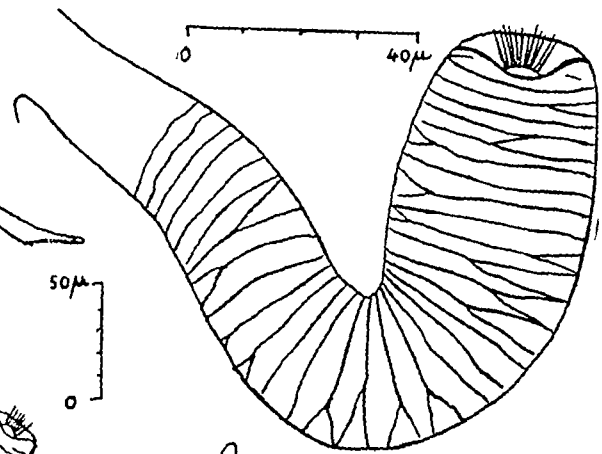

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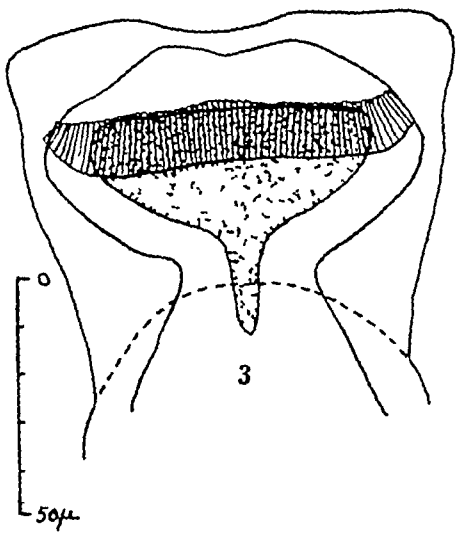

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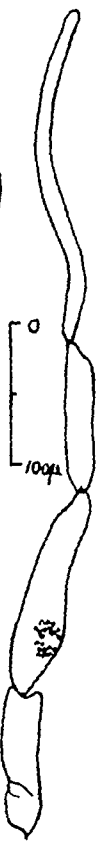

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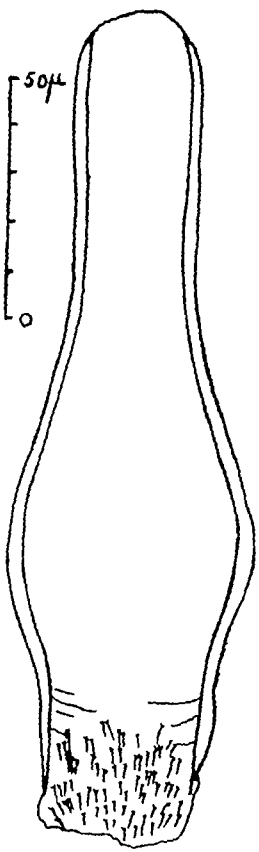

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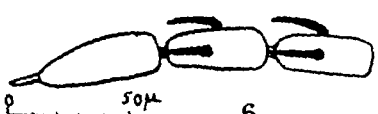

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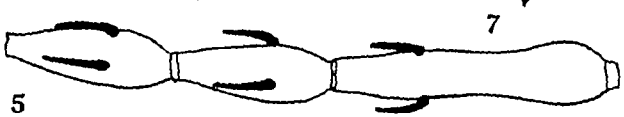

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4

Fig 1 Spermatheca, spermathecal ducts and furca
„ 2 Spermatheca
„ 3 Buccal cavity
„ 4 Wing

Fig 5 Antennal segments III–V
„ 6 Antennal segments XIV–XVI
„ 7 Palp
„ 8 Pharynx

Plate XXXIX

Phlebotomus hodgsoni n sp (♂)

Fig 9 Palp
„ 10 Antennal segments III and IV
„ 11 Antennal segments XIV–XVI
„ 12 Buccal cavity
Fig 13 Intermediate appendage and intromittent organ
„ 14 Pharynx
„ 15 Hypopygium
„ 16 Wing

Ind Jour Med Res, XX, 3, *January*, 1933

A NEW AFRICAN SANDFLY—*PHLEBOTOMUS TRANSVAALENSIS* N SP

BY

LIEUT-COLONEL J A SINTON, M D, D SC, I M S
(*From the Malaria Survey of India, Kasauli*)

[Received for publication, September 30, 1932]

THE female *Phlebotomus* described in this paper was received in a collection kindly sent by Mr B de Meillon from the Transvaal The specimen was labelled 'Tzaneen, Transvaal, S A December, 1931 B de Meillon'

Appearance in dry specimen

A very dark-coloured insect with distinct tufts of erect hairs on the dorsal surfaces of the abdominal segments These erect hairs are golden yellow, while the other hairs of the body are slightly browner The integument of all the body and legs is practically black and the pleuræ are little, if at all, lighter in colour The wings have dark brown, almost black, hairs and show a greenish iridescence The antennae are black with dark grey hairs There seem to be a few broad scales along the posterior border of the hind coxa The very long superior claspers (cerci) are a marked character

Appearances in stained and mounted specimens

The measurements and relative lengths of the different parts of the body are given in the Table

The *body*-length is about 3 75 mm The scars of the erect hairs on the dorsal surfaces of the abdominal segments are very evident but there are also many scars of recumbent hairs in the same areas

The *buccal cavity* (Plate XL, fig 6) has a well-developed pigmented area and the armature consists of about 50 long, narrow, contiguous teeth, set in a line with the concavity slightly backwards The *pharynx* (Plate XL, fig 7) does not show a very marked posterior dilatation The armature consists of a number of small teeth near the middle line and rows of very minute teeth laterally

The *antennae* (Plate XL, figs 3–5) The measurements and relative lengths are shown in the Table The geniculate spines are slender The knob on the end of segment XVI is smaller than usual

The *palps* (Plate XL, fig 2) Segment 3 is slightly shorter than 4, which is not usual in this sub-genus Newstead's spines number about 15

The *wing* (Plate XL, fig 1) is unfortunately incomplete

The *female hypopygium* (Plate XL, figs 8–10) The superior claspers (cerci) are very long and narrow, a feature not observed in any of the other Old World *Phlebotomus* (cf *P papatasii*, Plate XL, figs 11-12) The sternite of the 8th abdominal segment has a number of very stout, long bristles arising from its posterior border, a feature not noted in any other species The post-genital plate is a very elongated triangle, not the usual rectangle seen in other species The spermatheca (Plate XL, fig 10) is sausage-shaped and segmented

Differential diagnosis

This species has both erect and recumbent hairs on the dorsum of the abdomen The morphology of the cerci at once differentiates the female of this species from any of the other ones described The shape of the post-genital plate, the stout spines on the 8th abdominal sternite and the morphology of the spermathecae, buccal cavity and pharynx are of diagnostic importance

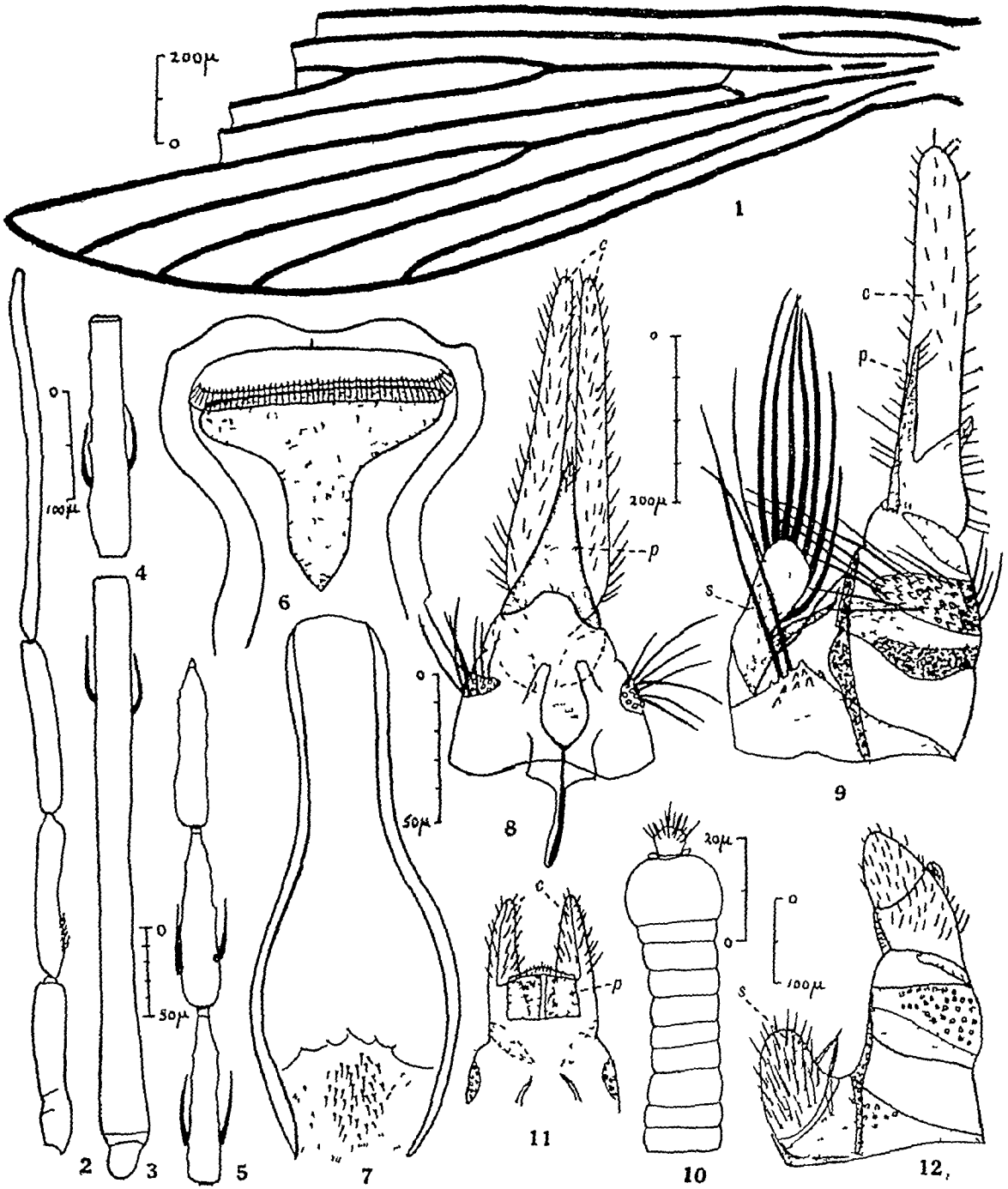
The male of this species has not yet been found It is proposed that the species, which belongs to the sub-genus *Sintonius* Nitz, be named *Phlebotomus transvaalensis*

TABLE

Phlebotomus transvaalensis N SP (♀)

	Structures	Length in mm	Remarks, relative lengths, etc
BODY	Head and clypeus	0 457	
	Thorax	0 900	
	Abdomen, proper	1 957	
	Superior clasper	0 443	
	Total length	3 75	
MOUTH	Labium	0 285	
	Pharynx, length	0 207	= 2 75 × breadth
	Pharynx, breadth	0 075	= 2 5 × narrow anterior part

Plate XL



Phlebotomus transvaalensis n sp (♀) (figs 1–10) (1) Wing (2) Palp (3) Antenna, segment III (4) Antenna, segment IV (5) Antenna, segments XIV–XVI (6) Buccal cavity (7) Pharynx (8) Hypopygium, ventral view with 8th abdominal sternite removed (9) Hypopygium, lateral view (10) Spermatheca

Phlebotomus papatasii (♀) (figs 11–12) (11) Hypopygium, ventral view with 8th abdominal sternite removed (12) Hypopygium, lateral view

Lettering —*p* post genital plate c cerci, *s* 8th abdominal sternite

TABLE—*concld*

	Structures	Length in mm	Remarks, relative lengths, etc
ANTENNA	Segment III	0 315	> IV+V
	Segment IV	0 123	
	Segment V	0 126	Formula = $\frac{2}{\text{III—XV}}$
	Segment VI	0 129	
	Segments XII–XVI	0 483	= 1 53 × III
	Total length	1 930	= 6 1 × III, 4 0 × XII—XVI
PALP	Segment 1	0 048	Formula 1, 2, 3, 4, 5
	Segment 2	0 114	Relative lengths 3 0, 7 1, 9 4, 10 0, 22 4
	Segment 3	0 150	
	Segment 4	0 160	= 1st+2nd
	Segment 5	0 336	
	Total length	0 808	
WING	Length	2 570	= 4 × breadth
	Breadth	0 343	
	α		$\frac{\beta}{\gamma}$ = 1 03
	β	0 470	
	γ	0 457	
HIND LEG	Femur	1 030	0 870*
	Tibia	1 230	1 014
	Tarsus, seg 1		0 457
	Tarsus, segs 2–5		0 643
	Total length		3 000 (Not including coxa and trochanter)

* Middle leg

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THE CHOLESTEROL CONTENT OF BLOOD IN INDIANS AND ITS SIGNIFICANCE IN JAUNDICE

BY

A C GHOSE, MB, BS,

Captain Kunwar Indrajit Singh Research Scholar

(From the Department of Surgery, Lucknow University)

[Received for publication, September 9, 1932]

THE cholesterol content of blood in a large number of different diseases has been found to have some significance from the ætiological, diagnostic, prognostic, and from the treatment points of view Of the pathological factors, cholelithiasis, diabetes, chronic nephritis, insanity, arterio-sclerosis, and of the physiological factors, pregnancy and the pre-menstrual phase of the menstrual cycle, have frequently been found to cause an increase in the blood cholesterol In leprosy, tuberculosis, anæmias and in acute infections the cholesterol content of the blood is usually low

The rôle of cholesterol metabolism in malignant diseases, pernicious anæmia, and in various forms of jaundice has also been considered by many workers Within the last twenty years many valuable contributions to the literature on this biochemical constituent of the blood have been made but the part it plays in the economy of the body still remains a subject for further investigation This paper deals essentially with the blood cholesterol in normal Indians, residing in the United Provinces, and also in the diseases of the liver and biliary tract accompanied by icteroid coloration of the conjunctivæ

The actual cholesterol estimations were performed on oxalated blood The alcohol-ether extraction method of Bloor (1916) was employed which yields rather higher values than the widely used technique of Wardell-Myers The standard solution was made of pure cholesterol dissolved in chloroform and the colour was produced by the treatment of acetic anhydride and sulphuric acid, after producing Leibermann-Burchard reaction the colour was matched with that of the unknown similarly treated in a Dubosq colorimeter

1 NORMAL CHOLESTEROL CONTENT

Before any right conclusions could be drawn from the investigations on the blood cholesterol in jaundice, it was found necessary to obtain the normal values of the blood cholesterol in the inhabitants of this part of the country

The investigations on the cholesterol content of the whole blood were done on 80 males and on 24 females who have had no pregnancy (Ghose, 1931) The average value of the blood cholesterol was found to be 146 mg per cent, the maximum value was 184 and the minimum was 130 The average of the plasma cholesterol was 140 mg per 100 c c calculated from the results obtained in 36 cases, the blood serum cholesterol in 42 cases gave 135 mg per cent as the average value—the lowest value being 110 mg and the maximum value was 180 mg per 100 c c The results obtained, indeed showed a wide range of normal values Such a wide range in normal values between 190 and 260 mg per 100 c c was also obtained by Bloor (1916) Henes (1913) found figures ranging between 110 and 182, and Gorham and Myers (1917) and Robinson (1929), who followed Leibermann reaction, obtained similar results Both Leibermann and Salkowaski's reactions in various methods of cholesterol estimations, yield a wide range of normal values Weston and Kent (1912), who used Salkowaski reaction, found the values between 117 and 297 mg per cent, which is really a very wide range

It is clear that the normal blood cholesterol values obtained by me by Bloor's method in Indians are rather lower than those obtained by Bloor in Western people Boyd and Roy (1928) also estimated the blood cholesterol in Indians in the vicinity of Calcutta and obtained an average value of 112 mg per 100 c c by Wardell-Myers' method, which is lower than that obtained in Europeans by this method Thus, we find that the normal values of the blood cholesterol in Indians yielded by the standard methods is about 40 mg per cent less as compared to the occidental standard

Estimations on blood cholesterol were made on 182 subjects, out of whom there were 60 strict vegetarians and 122 who took a mixed diet When the blood cholesterol values of individuals were compared, it was found that the blood cholesterol in pure vegetarians was slightly less by about 10 to 15 mg per cent than among those who took a mixed diet, but it was neither a constant feature nor did the cholesterol values cross the normal limits

The blood cholesterol was found to be fairly constant for any individual, it was estimated at different times in the day and for two to three days consecutively, but no appreciable changes were found

The immediate effects of lipoid diet, after fasting, on the blood cholesterol level was estimated in 12 healthy men after giving four eggs and half a seer of milk (Ghose, 1931) The blood cholesterol curve, obtained from a number of hourly investigations after the fatty diet, shows that there is a rise of the blood cholesterol level reaching to its maximum level of about 180 mg in

two-and-a-half hours time This rise in the blood cholesterol level is temporary in nature and usually comes down to normal in five to six hours after the food is taken The effect of diet on the cholesterol content of the blood has been shown by Bloor (1932), Gardner and Lander (1913), Fraser and Gardner (1910), and Gardner and Gainsborough (1928) and others Robinson (1929) has obtained similar results in cats, and mentions that Rothschild and Luden's figures prove the constant character of the cholesterol content of the blood in the experimented animals

The blood cholesterol was estimated during autumn, winter, spring and summer, the values of the cholesterol content of the blood of the same subject during each season was practically the same and no changes in the cholesterol content of the blood was noticed even during the hottest months (May, June, and July) Morse (1927) remarks that the low cholesterol content in Orientals is probably due to the effect of hot climate—a large amount of cholesterol being excreted by the secretions of the sebaceous glands, which might be responsible, with or without being associated with other factors, for the maintenance of the cholesterol content of the blood A seasonal variation in the blood cholesterol has, however, been mentioned by Currie (1924), the average values being much higher in the summer than in the winter, but Maxwell (1928) investigated this point and did not find any truth in it

The specimens of blood for the estimation of the cholesterol content were usually taken between 9 and 12 A M , when the subjects have had light diet in the morning In this way any error in the blood cholesterol values due to the supposed effect of starvation was eliminated, because Rothschild (1915) has mentioned the association of starvation with the raised blood cholesterol values

2 Variations in jaundice

On referring to the literature one finds that observations have been made by many workers on the cholesterol content of the blood in various forms of jaundice Previous workers usually dealt with the total cholesterol in the blood plasma but Gardner and Gainsborough (1930) have thrown a new light on the subject, showing the relationship of free cholesterol to its ester form in the blood plasma in the cases of hepatic and biliary diseases associated with jaundice

Observations on the blood cholesterol in hæmolytic jaundice have been made by McAdam and Shiskin, Muller and others, which show varying results In the present group of cases there was no case of hæmolytic jaundice, but all the cases of icterus in the group were either of catarrhal or of obstructive nature due to various causes

The cholesterol content of the blood was estimated in the cases of jaundice together with the determination of the icterus index of the serum, in order to

ascertain the relationship between cholestræmia and bilirubinæmia, if there be any

The normal values of the icterus index in Indians obtained by Ghose (1931) are between 3 and 6 and it was shown by him that when the icterus index rises above 10 there are usually signs of clinical jaundice Judd (1925), Milroy (1929) and Muller *et al* (1925) also give similar normal values and report that, with an icterus index of about 15, signs of clinical jaundice occur

TABLE I

Icterus index and the blood cholesterol in jaundice

Number of cases	Diseases	Jaundice	Icterus index	Blood plasma cholesterol mg per cent
5	Endemic ascitis	+	9 to 10	90 to 130
4	Cirrhosis of liver	+	9 to 20	90 to 145
10	Catarrhal jaundice	++	10 to 25	130 to 150
4	Cholecystitis	+	10 to 24	100 to 280
5	Cholelithiasis	++	10 to 40	220 to 330
6	Cancer of liver	++	9 to 45	80 to 130
2	Gumma of liver	++	30 and 65	100 and 130
2	Cancer of liver, gall bladder with gall stones	+++	40 and 50	335 and 260
3	Cancer of liver, pancreas and stomach	+++	80 to 120	80 to 130

RESULTS

There were 41 cases of jaundice in which the conjunctivæ were coloured yellow of various intensities in different conditions Five cases of endemic ascitis and four cases of cirrhosis of liver showed slight icteroid coloration of the conjunctivæ, the icterus index was raised but the blood cholesterol was normal in 4 cases and low in 5 cases There were 10 cases of catarrhal jaundice in which the conjunctivæ showed marked icteroid coloration, the icterus index was high and the blood cholesterol was within normal limits in all these cases

Of 4 cases of chronic cholecystitis and 5 cases of cholelithiasis which showed a slight tinge of icterus, the icterus index was high and there was an increase in the cholesterol content of the blood in all the cases except one in which the liver was extremely cirrhotic There were 2 cases of gall-stones complicated by the

carcinomatous changes in the liver and gall-bladder in which there was deep jaundice, even the skin showing slight icteroid coloration The icterus index was abnormally high and the blood cholesterol was increased

There were 2 cases of gummatous condition of the liver and 6 of carcinoma of the liver with deep jaundice In these cases the blood cholesterol was rather low and the icterus index high Three cases of cancer of liver, pancreas and stomach, with very deep jaundice without gall-stones, gave a very high value of icterus index and a low value of blood cholesterol Clinically, the jaundice at the beginning was of lemon colour, which gradually changed into reddish yellow and finally into dirty brown mixed with slight greenish tinge The cases had rapid fatal endings

Thus, the blood cholesterol in catarrhal jaundice is normal, while in obstructive jaundice due to gall-stones it is decidedly high This agrees with the results obtained by Rothschild and Rosenthal (1916), Campbell (1925), and Robinson (1929) who found highest values in jaundice with gall-stones Gardner and Gainsborough (1930) report that hyper-cholestræmia does occur in the biliary obstruction caused by stones and accompanied with jaundice, but the percentage of the total cholesterol, as ester, remains below normal In the cases of cancer of the liver, pancreas and gall-bladder, without gall-stones with deep jaundice, in which the blood cholesterol was not found to be increased, the results obtained essentially differ from those obtained by Rothschild, Rosenthal and Robinson and others who found high blood cholesterol in this condition

Summary

1 An investigation on the cholesterol content of the blood was made in healthy Indians The normal blood cholesterol average value is 146 mg per cent of the whole blood and 140 mg per cent of the blood plasma yielded by Bloor's method, which is about 40 mg per cent less than the European and American standards A wide range of normal values have also been found in Indians

2 In vegetarians and mixed dietarians the cholesterol content of the blood is practically the same A rich fatty diet after fasting gives rise to temporary increase in the blood cholesterol

3 The seasonal variations seem to have little effect on the cholesterol content of the blood

4 The changes in the cholesterol content of the blood plasma which occur in biliary and hepatic diseases show —

(*a*) that a low blood cholesterol with slight jaundice is indicative of either cirrhotic or carcinomatous changes in the liver

(*b*) that a frankly low plasma cholesterol with very high icterus index is indicative of malignant growth of the pancreas, gall-bladder and of the adjoining organs, causing obstruction in the flow of bile

(*c*) that a high blood cholesterol with jaundice is indicative of gall-stones causing obstruction, it may or may not be associated with a cancerous condition of the gall-bladder and the pancreas

In conclusion I offer my thanks to Professor R N Bhatia for his keen interest in the work The work was carried on during the tenure of a research fellowship

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THE SIGNIFICANCE OF THE BLOOD CHOLESTEROL IN THE SURGERY OF THE GENITO-URINARY SYSTEM

BY

A C GHOSE, M B , B.S ,

Captain Kunwar Indrajit Singh Research Scholar

(*From the Department of Surgery, Lucknow University*)

[Received for publication, September 9, 1932]

THE number of operations performed on the genito-urinary system are by no means few In spite of the clinical features of a case of urinary trouble for operation being good, in spite of a fair margin of safety assured by the blood urea estimation and urea concentration tests, a few cases die and in a few recovery is prolonged due to bacterial infections of the urinary tract The capacity of elimination of the urine remaining efficient, there must be some other factor which may be responsible for lowering the resistance of the body to the bacterial infections

McAdam and Shiskin (1925) were the first to point out that a low blood cholesterol is directly related to the urinary infections after operations and to a high rate of mortality due to sepsis Maxwell (1928), however, do not seem to have found any such prognostic significance of the blood cholesterol and blood urea ratio in the surgery of the genito-urinary system

With a view to finding out if the estimation of the blood cholesterol, as a routine method, be of some value in the selection of cases for operations on the genito-urinary system, an investigation was made on the cholesterol content of the blood plasma in cases necessitating—(*i*) nephrectomy for inflammations, multiple stones and tumours of the kidney, (*ii*) prostatectomy for the enlarged prostate, (*iii*) lithotomy for the calculous diseases of the genito-urinary tract, (*iv*) cystostomy for the removal of growths from the bladder, and (*v*) urethrotomy for the stricture of the urethra

METHODS

The blood cholesterol was estimated by Bloor's method (1916) The blood plasma cholesterol was determined in order to compare the results with those of the

previous workers on the subject The estimation of the blood urea was done by the method of Myers as described by Morse (1927), which is based on the principle of removing from the urea the ammonia formed by the action of the enzyme of *soya* bean or urease tablets, as in the method of Folin

Results

The normal value of the blood plasma cholesterol has been found to range between 110 mg and 180 mg per cent Such a wide range in the normal values has been obtained by many workers Maxwell (1928) therefore emphasized the fact that low blood cholesterol, i e , below 130 mg per cent, which is usually considered to be low, may be within the normal limits Owing to these elastic limitations in the normal blood cholesterol values, Gardner (1927) suggested that the blood cholesterol during disease should be compared to the normal blood cholesterol of that subject prior to the disease This idea, though excellent, is not practicable when the subject is first seen in the hospital as a patient McAdam and Shiskin (1923) considered 130 mg per cent of the blood cholesterol as the lowest limit of the normal value

1 Table I shows the results of the blood cholesterol estimations obtained in the cases in which nephrectomy was performed , out of 5 nephrectomies there was one death—the blood cholesterol in this case was normal In two cases the blood cholesterol was low, but the patients recovered after operation without any septic complications

Table I

Blood cholesterol in renal diseases in which nephrectomy was performed

Case number	Blood cholesterol mg per cent	Blood urea mg per cent	Disease	Remarks
19	140	50	Renal tumour	Recovered
22	120	45	Hydro nephrosis	Do
28	145	56	Do	Do
55	150	90	Do	Died of sepsis
63	120	60	Pyelo nephritis	Recovered

2 Table II The blood cholesterol was estimated in 17 cases of enlarged prostate in which 13 cases were operated upon One case (No 9) died and in two cases recovery was prolonged due to septic complications—the blood cholesterol was not below normal in any of these three cases There were 7 cases of enlarged prostate in which the blood cholesterol was below 130 mg per cent, and 5 out of these cases were operated upon, but there was no death

TABLE II

Blood cholesterol in enlarged prostate

Case number	Blood cholesterol mg per cent	Blood urea mg per cent	Operation	REMARKS
8	150	45	P	Recovered
9	140	90	P	Died
14	130	40	P	Recovered
25	135	56	P	Do
45	165	64	C and P	Do
47	120	35	C and P	Do
51	90	30	C	
52	120	35	C	
53	90	50	C and P	Do
54	130	25	C	
57	90	42	C	
58	120	20	C and P	Do.
59	125	32	C and P	Do
60	145	35	C	
61	130	21	C and P	Do
62	180	28	C and P	Do
63	120	16	P	Do

C =Cystostomy P =Prostatectomy

3 In Table III the blood cholesterol figures are shown in the 'calculous diseases' of the genito-urinary system Out of 34 lithotomies, there were 5 deaths

due to septic complications, in which 2 cases had low blood cholesterol There were 6 other cases in which the blood cholesterol was below 130 mg per cent, but the cases made a rapid recovery after the operations

TABLE III

Blood cholesterol in the calculous diseases of the genito-urinary system

Case number	Blood cholesterol mg per cent	Blood urea mg per cent	Operation	REMARKS
1	140	45	Cystostomy	Recovered
2	160	50	Do	Do
3	130	35	Uretero lithotomy	Do
4	120	30	Do	Do
5	124	20	Pyelo lithotomy	Do
6	130	40	Do	Do
7	170	47	Do	Do
10	160	30	Cystostomy	Do
11	130	42	Pyelo lithotomy	Do
12	160	28	Do	Do
13	120	23	Cystostomy	Do
16	125	204	Do	Died of sepsis
17	190	65	Pyelo lithotomy	Recovered
18	170	25	Do	Do
20	130	20	Cystostomy	Do
21	145	45	Urethro lithotomy	Do
23	120	60	Pyelo lithotomy	Do
24	120	65	Do	Died of sepsis
27	145	60	Cystostomy	Died of cystitis
29	150	34	Do	Recovered
30	160	24	Do	Do
31	130	52	Pyelo lithotomy	Do
48	150	41	Nephrostomy	Do
32	130	48	Do	Do
50	180	24	Uretero lithotomy	Do
33	145	42	Excision of bladder	Do
56	90	40	Pyelo lithotomy	Do
34	160	65	Cystostomy	Died of cystitis
35	110	21	Do	Recovered
37	100	50	Do	Do
43	140	35	Uretero lithotomy	Do
44	145	28	Do	Do
46	160	45	Pyelo lithotomy	Do
49	165	56	Nephrostomy	Died of sepsis

4 Table IV There were 8 cases of retention of urine due to the stricture of the urethra, in which external urethrotomy was performed in 7 cases There was one death (No 15), in which the blood cholesterol was normal, and 3 other cases, in which the blood cholesterol was low, recovered without any complication

TABLE IV

Blood cholesterol in the cases with the stricture of the urethra in which external urethrotomy was performed

Case number	Blood cholesterol mg per cent	Blood urea mg per cent	Operation	Remarks
15	140	89	Urethrotomy	Died
26	150	50	Do	Recovered
36	120	56	Do	Do
38	150	44	Do	Do
39	90	50	Do	Do
40	100	30	Do	Do
41	140	69	Do	Do
42	135	40	Unfit for operation	

The results obtained in Tables I, II, III and IV show no evidence of a prognostic significance of a low blood cholesterol in the surgical diseases of the genito-urinary system in which there is no other contra-indications for an operation

Discussion

McAdam and Shiskin (1925) reported on 88 cases of enlarged prostate and found low blood cholesterol in 18 cases, 16 of which died of pyelo-nephritis and only 2 recovered Of the remaining 70 cases which yielded blood cholesterol values above 130 mg per cent, only 11 died and one as a result of ascending urinary infection They also noted that in cases with low blood cholesterol there were no clinical contra-indications for operation Maxwell (1928) reported on 19 cases of enlarged prostate and out of them 5 cases gave blood cholesterol values below 130 mg per cent Of these cases, 3 recovered and 2 died of pyelo-nephritis The remaining 12 cases of Maxwell had normal blood cholesterol, 5 cases were not operated upon owing to the presence of bad operative risks, and the remaining 7 cases were operated upon and of these 2 died and 5 recovered

In the malignant diseases of the prostate McAdam and Shiskin do not consider the plasma cholesterol of any significance, because in malignant diseases the blood cholesterol is frequently found to be increased as mentioned by Luden (1918) Brunton (1927) on the other hand obtained very variable results in such cases, so also did Maxwell (1928) In 3 cases of carcinoma of the bladder Maxwell found low blood cholesterol Supra-pubic cystostomy was performed on these patients

with the result that one died of peritonitis, one of uræmia and the third improved There was one case of papilloma of the bladder with normal blood cholesterol which died after operation

Maxwell also reported of 3 cases of retention of urine due to the stricture of the urethra , two cases had a low plasma cholesterol which recovered after obstruction was removed, while one case with normal blood cholesterol died due to pyelitis and uræmia

Summary and conclusions

1 The cholesterol content of the blood was estimated in 62 cases suffering from the surgical diseases of the genito-urinary system The results obtained in the course of the present investigations do not point to any significant utility of the blood cholesterol determination, as a routine method, which would forecast the prognosis in a case of urinary disease

2 A low blood cholesterol has no prognostic significance in operated cases of urinary obstruction, and there is no justification to refuse an operation on a sufferer from urinary obstruction, merely because on one occasion previous to the proposed operation a low blood cholesterol had been reported

3 Blood urea below 50 mg per cent assures a fair margin of safety, and no direct or indirect relation of the blood urea to the cholesterol content of the blood has been found

In conclusion I take this opportunity of thanking Professor R N Bhatia for his keen interest in the work and to Dr A Hameed for help in the preparation of the paper

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DETERMINATION OF H ION CONCENTRATION OF BODY-FLUIDS AND TISSUES WITH GLASS-ELECTRODE

BY

G SANKARAN, MB, BS,

Nutritional Research Laboratories, I R F A, Coonoor, South India

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THE importance of H ion concentration in the tissues is now being recognized in all branches of physiology and pathology The difficulties attending the use of the hydrogen-electrode and its limitations, as well as those of colorimetric methods of pH determination have, however, been a great hindrance to progress in this branch of research With the introduction of the glass-electrode (Brown, 1924) these difficulties have, to some extent, been overcome It is now possible, after familiarity with the use of this electrode has been acquired, to take pH readings of minute quantities of any tissue or body fluid within a couple of minutes and with an accuracy and reliability unobtainable by older methods

Principle of the glass-electrode

The principle of the glass-electrode is that when a glass-membrane of about 25μ thickness separates two liquids differing in their pH values, an electrical potential is developed whose value is a function of the difference of the pH values If we keep a constant pH, such as is given by any buffer solution, on one side of the membrane then the pH of any unknown on the other side can be determined by means of a potentiometer which indicates the potential developed A difficulty, however, lies in the fact that on account of the high resistance of the glass-membrane—of the order of 100 megohms—the current generated by the potential is so slight that it cannot be detected by even the most delicate galvanometers This is overcome by the use of electrometers which require very careful insulation and shielding from electrical disturbances in the atmosphere In the typical Lindemann electrometer a needle is kept stationary between two metal plates each having a charge, opposite in sign, of about 12 to 20 volts When the potential developed on either side of the membrane is led to the needle a deflection of the needle occurs The needle can then be brought back to the original position by sending in a potential in the opposite direction from a standardized potentiometer For these manœuvres a couple of non-polarizable calomel-electrodes, which lead off the current from either side of the glass-membrane, are employed, the potential

necessary for the restoration of the electrometer-needle to its original position is indicated on the dial of the potentiometer and represents the value of the electrical potential developed in consequence of the difference of the pH values on either side of the glass-membrane

The equipment

The equipment, as used in this Laboratory, is that supplied by the Cambridge Scientific Instrument Company, slight modifications have been introduced which add to its convenience

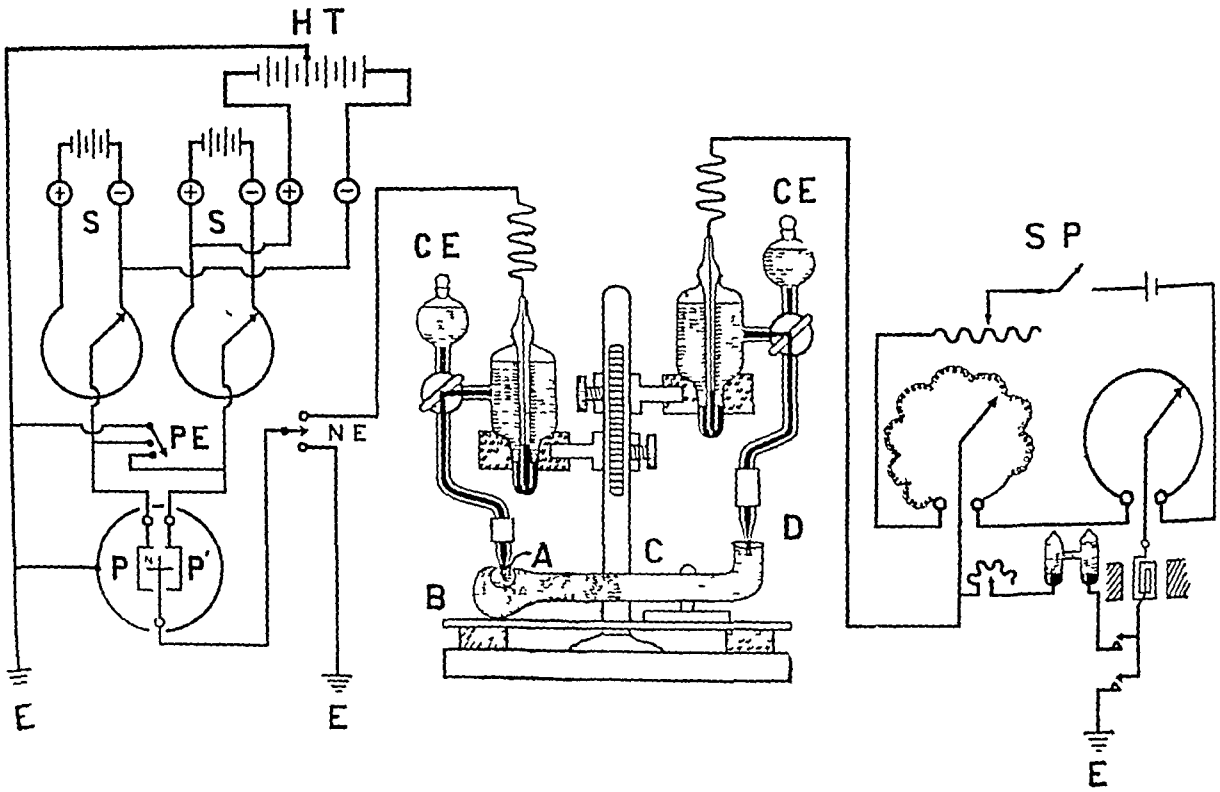

Fig 1

H T High tension battery
S and S¹ Potentiometric system to regulate voltage on plates
P E Switch for earthing plates
N E Switch for earthing needle
P and P¹ Plates of electrometer
N Needle of electrometer
E Earthing terminal
C E Calomel electrodes
A Cup of glass electrode
B Bulb of glass electrode
C Body of glass electrode
D Open end of glass electrode
S P Standard potentiometer

The Lindemann electrometer (Lindemann *et al*, 1924) is a robust unit mounted on a conveniently-sized base, the needle being focussed under a microscope having a low power objective and a high power eyepiece, the latter fitted with a micrometer scale The plates of the electrometer (P P¹ Fig 1) are charged from a battery of cells (H T, Fig 1) Either an accumulator battery or a high

tension-radio-battery of dry cells may be used for this purpose, the latter is the more convenient The needle (N Fig 1) of most electrometers is steady only up to some particular voltage on the plates Any higher voltage makes the needle unsteady Also the sensitivity of the electrometer increases with the voltage on the plates, so that it is necessary to have the voltage as high as possible and yet keep the needle steady This is achieved by gradually increasing the charge on the plates until the needle just begins to tremble when in the earthed position, and then gradually decreasing the potential by means of an opposed potentiometer system (S S^1, Fig 1) as shown in the circuit diagram In the circuit shown a 12-volt charge, from H T on each plate, is opposed by a 4-volt one from S and S^1 so that the charge can be varied down to 8 volts at will Two switches are also shown P E, which enables the plates to be earthed and N E, which enables the needle to be earthed In Fig 1 the location of the connections of the batteries and of the earthing switches, as well as that of the dials, in the unit supplied by the makers, are indicated

Before setting up the electrometer for work it is necessary to note whether any mechanical vibration from the floor of the laboratory effects the needle of the electrometer The equipment can safely be placed on a laboratory table provided the floor be steady, but even then persons walking near to it may cause unsteadiness of the delicate needle To overcome this trouble it is best to place the electrometer on a wall-bracket of marble or slate, fitted into the wall by means of rigid struts Even then care has to be taken not to lean on the bracket while taking a reading, otherwise mechanical vibrations from the body may cause the needle to tremble When the needle is earthed its position under the microscope marks the zero point when it is unearthed the potential from either side of the glass-membrane is led on

The non-polarizable calomel-electrodes (C E, Fig 1) that lead off the current from either side of the membrane are of the usual saturated-potassium-chloride type The manufacturers supply these with ground-glass tips, these are clumsy and apt to drop off at inconvenient moments and to break the thin glass-membrane A very convenient device is to attach finely drawn-out glass nozzles (seen at A and D Fig 1) filled with saturated-potassium-chloride-agar, to the tips of the calomel-electrodes These nozzles are of the size and shape indicated below (Fig 2) —

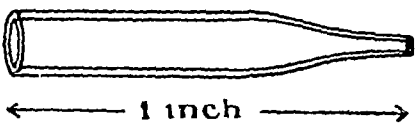

Fig 2

The method of filling them, with the saturated-potassium-chloride-agar, is as follows They are first cleaned in chromic acid solution and then washed in

several changes of distilled water They are then dropped vertically—narrow end upwards—into a hot, 2 per cent solution of agar in saturated-potassium-chloride solution In this way the agar completely fills the nozzles without the inclusion of air-bubbles The agar solution is then allowed to set and when nozzles are needed they are cut out of the jelly and attached with rubber-tubing to the calomel-electrode To prevent the agar drying, when the nozzles are not in use, their tips are lowered into conveniently placed vessels containing a saturated solution of potassium chloride With care a pair of such nozzles should last a couple of months of regular use

The potentiometer set (S P, Fig 1) can be of any make that is sensitive to one millivolt A dry cell can be used but it must be standardized every day with a standard Weston cell

The glass-electrode used in this Laboratory is a modification of Mrs Kerridge's pattern (1925) and is shaped as shown in Fig 1 These electrodes should never be purchased ready-made but should be made by the individual worker for himself in sizes convenient for different kinds of work They are made of special Corning glass-tubing, No 015 It is advisable to keep a stock of this glass-tubing, of various diameters, in hand so as to acquire skill in blowing the electrodes for oneself, for the thin glass-membrane is likely to break even under slight strain and frequent renewals are needed The simplest way of making them is to take a piece of the glass-tubing about 5 or 6 inches long and of the required diameter (usually from 5 to 12 mm), seal one end in the flame and blow a bulb (B, Fig 1) of the required dimensions at the sealed end, then carefully heat, with a pointed flame the side of the bulb and sharply suck in the heated portion to form a spherical cup (A, Fig 1), finally the open end (D, Fig 1) is bent up half to one inch as shown in the figure The proper thickness of the film forming the cup is a matter of great importance, for when it is more than 25μ thick the potential per unit pH is far short of the theoretical 57 7 mv at 18°C Before use the electrode is cleaned with strong alkali, followed by chromic acid and repeated washings in several changes of distilled water Finally, it is soaked in distilled water for at least 24 hours The glass-electrodes are then tested with known buffers, say, 6 00 to 8 00 pH, and those in which the glass-membrane is too thick (i e , give values below 54 mv per unit pH) are rejected

A glass-electrode to be satisfactory must first be standardized The method adopted for such standardization is to prepare, with double distilled water, several buffers made up with potassium-dihydrogen-phosphate and sodium-hydroxide (Clark, 1920), and of pH values ranging from 6 00 to 8 00 With these as standards inside, and N/10 hydrochloric acid outside the electrode-cup, the potentials given are read off The values in millivolts are then charted on graph paper when, if the electrode be satisfactory, the increasing values fall on a straight line The graph so prepared is the standard graph for the particular electrode, it cannot be used

with any other It is easy from this graph to read off at a glance the pH of a tissue or tissue fluid which has given a certain potential It is always necessary to test the electrode with these buffers each day before starting the day's work , and if the readings are below or above the figures given by the graph, correction has to be made If, for example, the graph indicates that the electrode gave a reading of 350 millivolts for a pH of 7 00 and it be found, on any subsequent day, that it gives a reading of 355 millivolts for the same pH then 5 millivolts has to be subtracted from all readings obtained on that day so as to enable the graph to be used for conversion into pH values If, on the other hand, the electrode gives a reading of 345 millivolts for the same pH then 5 millivolts has to be added to all readings on that day Another, but more tedious, method is to prepare a new graph each day

For the routine work of this laboratory it has been found convenient to have electrodes varying in capacity from 5 c c down to 2 drops The most useful size is one that holds 10 drops or about half a c c

Technique of taking a reading.

Mrs Kerridge (1925) has stated that it is possible to take readings when the cup is only half full of the material under test This is so if the cup be of a capacity not exceeding 10 drops But when it exceeds this capacity very erratic potentials are obtained with anything less than a completely filled cup A typical set of results, as given with a cup about 2 c c capacity when it contains varying amounts of minced liver tissue, is as follows —

	Millivolts	pH	Millivolts	pH
3 drops	212	4 61	185	4 14
10 „	240	5 09		
1 c c	263	5 49	232	4 96
1 5 c c	254	5 34		
Full	302	6 16	302	6 16

This curious phenomenon can be explained thus The potential developed on either side of the glass-membrane is a function of the difference in pH on either side of it and is equal to the difference in the total charges of H ions in contact with each side of the membrane If on one side of the membrane there are a certain number of H ions which remains constant, and on the other side there is a test-material of a certain pH which is in contact with the membrane over different areas

at different times of taking the reading, then the resultant charge due to H ions on either side of the membrane will differ Working with electrodes of small capacity, say 5 drops, we get steady potentials with 2 or 3 drops of test-material, because in these circumstances, the fluids, due to capillary action, creep up the surface of the cup which being of small size is completely covered by them If, however, the cup be of a larger capacity its surface is not completely covered by the fluid under test and the potentials vary accordingly It is, therefore, advisable to have electrodes of a capacity suited to the amount of material available for test As previously stated those of 10 drops capacity are of most general use Those of very small capacity, say 2 to 5 drops, are difficult to make as the membrane has to be sufficiently thin , this is difficult to ensure when the cup of the electrode is of small size

Whatever be the capacity of the electrode the method of taking a reading is the same Rinse the cup once or twice with the test-material Wipe the outside of the bulb and the stem of the electrode thoroughly with dry filter paper or cloth (this procedure is necessary in order to prevent any layer of moisture on the outside of the electrode acting as a conductor) Carefully fill the cup of the electrode with the test-material Fit the electrode to a convenient wooden holder by means of a clip Place it in position underneath the calomel-electrodes and by means of the rack and pinion lower the latter, one into the cup and the other into the N/10 hydrochloric acid solution (*vide* Fig 1) With the needle in the earthed position the reading of the micrometer-scale is noted The needle is then unearthed (i e, charged), when it is seen to move along the micrometer-scale By adjusting the potentiometer-dial the needle is brought back to the original position To make sure that it has been brought back to the identical position the needle is repeatedly earthed until one is certain that there is no deflection

Method of taking pH of tissue fluids and tissues.

It is easy to take the pH of blood, urine or other fluids , all that has to be done is to fill the cup of the electrode with the fluid to be tested But in the case of tissues the material has to be pulped in order that it may come into contact with the inside of the cup The glass-electrode as at present made cannot be used for measuring pH in the living body The tissue has to be removed from the body and finely minced or pulped, the cup of the electrodes being then filled with the pulped material A number of experiments were carried out in order to see whether the degree of mincing gives rise to any variation of pH It was found that the pH remains constant whether the material was minced coarsely or finely or ground into fine paste in an agate mortar, always provided that it was in contact with the interior of the cup at every part Further, it was found that slight dilution with distilled water has no effect on the pH , this is well known to be so when the material under test is blood or urine,

Effect of time of removal from the body on the pH of tissues

Several experiments were carried out with the object of determining whether the pH of any given tissue varied with the length of time which had elapsed since the removal of the tissue from the body A certain mass of tissue was minced up and portions of it were examined at various intervals of time The results of two such experiments are given below , the examinations being made at laboratory temperature (about 20°C) and at about 0°C (i e , at cold storage chamber temperature) —

Time	MINCED LIVER TISSUE			
	21° CENTIGRADE		0° CENTIGRADE	
	Millivolts	pH	Millivolts	pH
On removal from body	302	6 16	302	6 16
1 hour after death	302	6 16	302	6 16
2 hours after death	302	6 16	302	6 16
3 „ „ „	302	6 16	302	6 16
4 „ „ „	300	6 12	302	6 16
5 „ „ „	298	6 08	302	6 16
24 „ „ „	258	5 41	302	6 16
26 „ „ „	252	5 31	302	6 16
48 „ „ „	242	5 23	302	6 16

Time	MINCED CHEST MUSCLE			
	20° CENTIGRADE		0° CENTIGRADE	
	Millivolts	pH	Millivolts	pH
On removal from the body	285	5 86	285	5 86
1 hour after death	285	5 86	285	5 86
2 hours after death	282	5 80	285	5 86
3 „ „ „	281	5 79	285	5 86
4 „ „ „	281	5 79	285	5 86
5 „ „ „	281	5 79	285	5 86
24 „ „ „	280	5 77	285	5 86

From these results it is evident that there is

(*i*) no change in the pH up to 2 days at 0°C,

(*ii*) no change up to 2 hours at room temperature, but there is

(*iii*) a definite change after 3 to 4 hours at laboratory temperature, probably due to alterations brought about by enzymes or bacteria

As we can easily make pH determinations of several minced tissues within an hour of their removal from the body, the method above outlined has proved to be sufficiently reliable for our work These results, however, apply only to tissues removed from animals that have been killed We have obtained evidence that when animals have died a natural death changes in pH of the tissues occur more rapidly

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THE PHARMACOLOGICAL ACTION OF 'THEVETIN'—A GLUCOSIDE OCCURRING IN *THEVETIA NERIIFOLIA* (YELLOW OLEANDER)

BY

LIEUT-COLONEL R N CHOPRA, M A, M D (Cantab), I M S,

AND

B MUKERJEE, M B (Cal)

(*From the Department of Pharmacology, School of Tropical Medicine and Hygiene, Calcutta*)

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Thevetia neriifolia (*Cerbera thevetia*), N O *Apocynaceæ* is known as yellow or exile or bastard oleander It is known as 'Pila-kanir' in Hindi and Gujrati, 'Kokilphul' or 'China karab' in Bengali and 'Pachhai alasi' in Tamil It is a large tree commonly met with in the plains all over India and is widely grown in gardens for its beautiful yellow flowers The plant was originally a native of the West Indies but has been completely naturalized in India It is about 12 feet high with linear, lanceolate leaves about 5 inches long and ¼ inch broad and has large yellow, bell-shaped flowers 3 inches in length All parts of the plant abound in a milky juice The fruits are globular, light green, about 1½ by 2 inches in diameter and contain a single nut, light brown in colour and of a peculiar triangular shape Each nut contains 2 pale yellow seeds These seeds have long enjoyed a reputation of being highly poisonous and have been very commonly employed for suicidal and homicidal purposes In Bengal both the whole fruit and the seeds have been used by women as abortifacients Of late years the seeds have come into somewhat extensive use in some parts of the Bombay Presidency as a cattle poison The plant is not used very much in therapeutics because of its highly toxic properties, but an alcoholic preparation of the bark is sometimes used as an anti-periodic in Hindu medicine, 30 to 60 drops of this preparation (1 in 5) act as a purgative and as an emetic

Chemistry of yellow oleander

De Vrij (1881, 1882, 1884) obtained from the kernel of the seeds 57 per cent of a limpid, almost colourless oil by extraction with benzol, which had a density of 0 9148 at 25°C and which solidified at 13°C After extraction of the oil he obtained

a crystalline white glucoside (yield about 4 per cent) to which he gave the name 'thevetin' The same glucoside was found in the bark of the plant Warden (1882) referred to a principle in the seed which gives a blue colour with hydrochloric acid and a second toxic principle which is more powerful than thevetin With the exception of a few colour reactions very little detailed study about the constitution of the glucoside, its melting point and other chemical properties appears to have been carried out by these workers B B De and A P Choudhuri (1919) isolated the glucoside 'thevetin' in a pure condition This was obtained in the form of cubes, it had a melting point of 189°C—190°C and decomposed at 205°C The molecular weight was also determined by the cryoscopic method, and from the results of combination they gave it the formula—$C_{72}H_{121}O_{36}$ The non-sugar product of hydrolysis of the glucoside was also isolated in an amorphous condition and was named 'thevetidin' This product is stated to be more toxic than thevetin These workers also remarked that the glucoside 'thevetin' loses its toxic power on re-crystallization, giving a filtrate which on evaporation is obtained as an uncrystallizable brown mass possessing more toxic power If so, it appears that during the process of re-crystallization some inter-molecular change occurs in the glucoside which is not equivalent to hydrolysis but which gives a toxic filtrate Ayyar (1928) isolated thevetin and described some of its chemical and physical properties He obtained 'thevetin' in glistening white plates softening at 190°C and finally melting at 215°C

Isolation of the glucoside —The following method for the isolation of the glucoside of yellow oleander was followed in our laboratory From 2,175 g of the kernel of the seeds of yellow oleander the oil was partially removed by expression and the residue extracted with hot 92 per cent alcohol After removing a large portion of the spirit by distillation the remainder was distilled under reduced pressure and at temperatures not above 70°C The dark brown mass containing much oil was washed three times with low boiling petroleum ether The residue was acid in reaction It was treated with precipitated chalk and then extracted with boiling absolute alcohol The alcoholic extract was concentrated to 200 c c and poured with vigorous stirring into a large volume of sulphuric ether in which the glucoside is insoluble In this way the last traces of oily and fatty substances were removed The glucoside crystallized as rectangular plates when slowly cooled from an alcoholic solution in a fortnight The crystals were washed with a very small quantity of water and the first washings which were coloured were discarded It was re-crystallized from aqueous alcohol (4 1), and was obtained in beautiful white rectangular plates, the length being 4 to 5 times that of the breadth The glucoside is sparingly soluble in cold water but freely soluble in hot water, from which it does not separate (limit $\frac{1}{2}$ per cent) It is freely soluble in dilute alcohol (50 per cent), the solubility decreasing with the concentration of the spirit It is insoluble in ether and chloroform After shaking with ethyl acetate and amyl

alcohol for a long time the glucoside is removed partially and crystallizes in an irregular system of plates From rectified spirit it separates as hexagonal plates with smooth and round corners The m p is found to be 189°C —190°C With strong H_2SO_4 it gives an ochre yellow colour turning red in about 12 hours, when heated with resorcinol and hydrochloric acid a pink colour is obtained (*J C S Abs*, 1923, p 1023) The glucoside is hydrolysed by dilute H_2SO_4 and HCl, the product reducing Fehling's solution as well as ammoniacal silver nitrate solution The non-crystalline brown residue, which is left after crystallization of the glucoside, gives the pseudo-indican colour reaction It also reduces Fehling's solution both before and after hydrolysis showing the presence of reducing as well as glucosidal substances An attempt is being made to isolate the second toxic glucoside reported by De and Choudhuri (*loc cit*)

Pharmacological action —In our series of experiments the effect of the drug was studied on frogs, rabbits and cats In toxicity experiments white mice and guinea-pigs were also used Cats weighing between 2 and 3 kilos were anæsthetized with chloralose (0 1 g per kilo body-weight), urethane (1 8 to 2 g per kilo body-weight) supplemented by ether, whenever necessary, was used in those experiments in which the effects on the respiratory system were investigated

Toxicity —The glucoside has been known to be highly toxic for a long time In order to determine the exact toxicity its effects on various organisms were tested On *Paramœcium caudatum* thevetin in concentrations ranging from 1 in 10,000 to 1 in 100,000 does not produce any inhibition of movements or death of the organisms The helminths obtained from the intestinal tracts of cats and dogs are kept in strong solutions of the glucoside but no toxic effects are observed Frogs, however, show definite poisoning effects The glucoside in doses of 0 2 mg causes the heart to slow down considerably and it ultimately stops in systole With fatal doses the toad shows a series of symptoms which were quite characteristic The animal, within five minutes of injection, shows quicker and deeper respirations and after 15 minutes the animal, when placed on its back, is unable to turn over The heart beats progressively slow down from the beginning Injection of atropine before administration of the drug rendered the slowing of the heart less marked The minimum lethal dose in these amphibia varies from 0 15 to 0 3 mg per kilo body-weight

White mice weighing from 10 to 20 g are given injections of thevetin in the tail vein With doses of 0 1 mg per gramme death occurs almost immediately With doses varying from 0 04 to 0 05 mg per gramme death occurs within five minutes General excitability and tremors of the head are noticed and sometimes paralysis of the hind limbs are seen before death The lethal dose varies from 0 03 to 0 04 mg per gramme

Guinea-pigs weighing from 400 to 600 g are given injections of thevetin subcutaneously and intraperitoneally Within a few minutes after subcutaneous

injection of the drug in toxic doses, the animal shows signs of being unable to keep the head in position There is a tendency for it to lie on one side The respiration is hurried and becomes quicker and deeper The motor excitability is slightly increased as evidenced by occasional twitchings of the whole body After some time, the animal shows a tendency to lie on its side or flat on its abdomen with the front limbs abducted Inco-ordination, especially of the hind limbs, is observed which sometimes ends in paralysis During the last stages convulsions are noticed, sometimes spontaneously and sometimes on slight external stimulation Involuntary passage of urine and fæces at these stages is not uncommon Death occurs from 15 to 40 minutes according to the dosage injected The lethal dose varies from 0 015 mg to 0 024 mg per kilo body-weight of guinea-pig subcutaneously Intraperitoneally thevetin in the same dosage as the subcutaneous causes death within 5 to 10 minutes of the injection The lethal dose works up to 0 01 mg per kilo by this route

Toxicity of thevetin was also determined on cats Thevetin dissolved in dilute alcohol was administered by means of a stomach tube in doses of 0 1 g , 0 12 g , 0 14 g , 0 15 g , and 0 2 g , per kilo body-weight to a series of cats weighing from 2 to 3 kilos The first animal receiving the smallest dose showed no appreciable effect whatsoever except slight salivation, hurried respiration and restlessness The second animal had retching movements , after half an hour it vomited segments of a tapeworm and appeared to be in a condition of extreme restlessness Breathing became hurried in 40 minutes and the mouth was filled with sticky saliva which clung to the jaws After about an hour the animal became quiet, the salivation decreased and in an hour-and-a-half completely stopped The respiration, however, remained hurried, the animal showed no inclination to move and tried to lie down quietly , death occurred eight hours later The third animal which was given 0 14 g per kilo body-weight showed within 20 minutes profuse salivation producing the same sticky saliva The respiration became accelerated and vomiting occurred twice or thrice Within an hour-and-a-half, a condition of coma set in and the animal died after about two hours No paralysis was noticed but spasms were found to occur in the limb muscles Animals receiving doses higher than 0 14 g showed almost the same train of symptoms as that occurring in the third cat but death followed much earlier

Post-mortem examinations of these animals did not show any congestion of the mucous membrane of the stomach, nor any signs of irritant poisoning as is reported in case of poisoning by *Nerium odorum* The heart stopped in diastole and organs like the kidneys, spleen, intestines, etc , were quite normal The visceral vessels were all found congested

Local action —The glucoside has no irritant action on the skin Instillation of a 1 per cent solution into the eye of a rabbit or a cat does not produce much local

irritation of the conjunctiva nor any anæsthesia of the cornea An injection of a 2 per cent solution into the thigh muscles of a cat sets up a mild congestion but no tissue necrosis or œdema is evident at the spot It is readily absorbed from the tissues

Alimentary system —The effect of the glucoside on the intestines was studied in cats under chloralose anæsthesia, as this anæsthetic has been found to have less effect on the automatic movements of the gastro-intestinal tract than most of the other anæsthetics in use Graph III, *B*, shows the result of an intravenous injection of 0 5 mg of thevetin in an intact animal, the peristaltic movements being recorded with a Jackson's enterograph It will be seen that there is a well-marked stimulation of the plain muscles of the intestine This stimulant action is at least partly due to peripheral stimulation of the nerve-endings of the vagus, as the effect disappears to a very great extent after the vagal endings are paralysed with atropine Stimulation of the plain muscles of the intestine is also partly responsible for the increase in tonicity

The effect of the alkaloid on the muscular activity of isolated pieces of ileum was studied in a Dale's uterine bath Both the tone and the movements of the gut were definitely stimulated by dilutions as low as 1 in 100,000 to 1 in 200,000 (Graph III, *D*) This shows that the drug has probably a direct muscular stimulant effect

Digestive enzymes —Thevetin even in high concentrations has little action on the digestive enzymes The peptic digestion is not affected in concentrations of 1 in 1,000 , the pancreatic digestion is not touched

Respiratory system —Graph III, *F*, shows the record of tracheal pressure with a Marey's tambour in a cat under urethane An injection of 0 25 mg of thevetin usually produces no change in respiratory excursions With a bigger dose, such as 0 5 to 0 75 mg , there is a tendency for the respiration to become slightly slower, but this seems to be only a temporary effect due to central vagal inhibition This effect is not observed after section of the vagi in the neck With toxic doses the respiratory tracings show a good deal of variations , this is probably secondary to the circulatory disturbances brought about by the drug to which reference will be made later When the vagal endings are paralysed with atropine an injection of thevetin shows a slowing of the respiration This is probably due to the sympathetic action getting the upper hand (Graph III, *G*)

The action of the drug on the bronchioles was tested by recording the intra-pleural pressure by means of a canula introduced through the ribs into the pleural cavity The animals were kept under artificial respiration from a mechanical pump with regulated number of strokes per minute so that the quantity of air pumped into the lungs remained quite constant Thevetin in 0 25 mg doses does not produce any appreciable change in the intra-pleural pressure records When the animal is given an injection of a toxic dose of the drug, however, certain changes

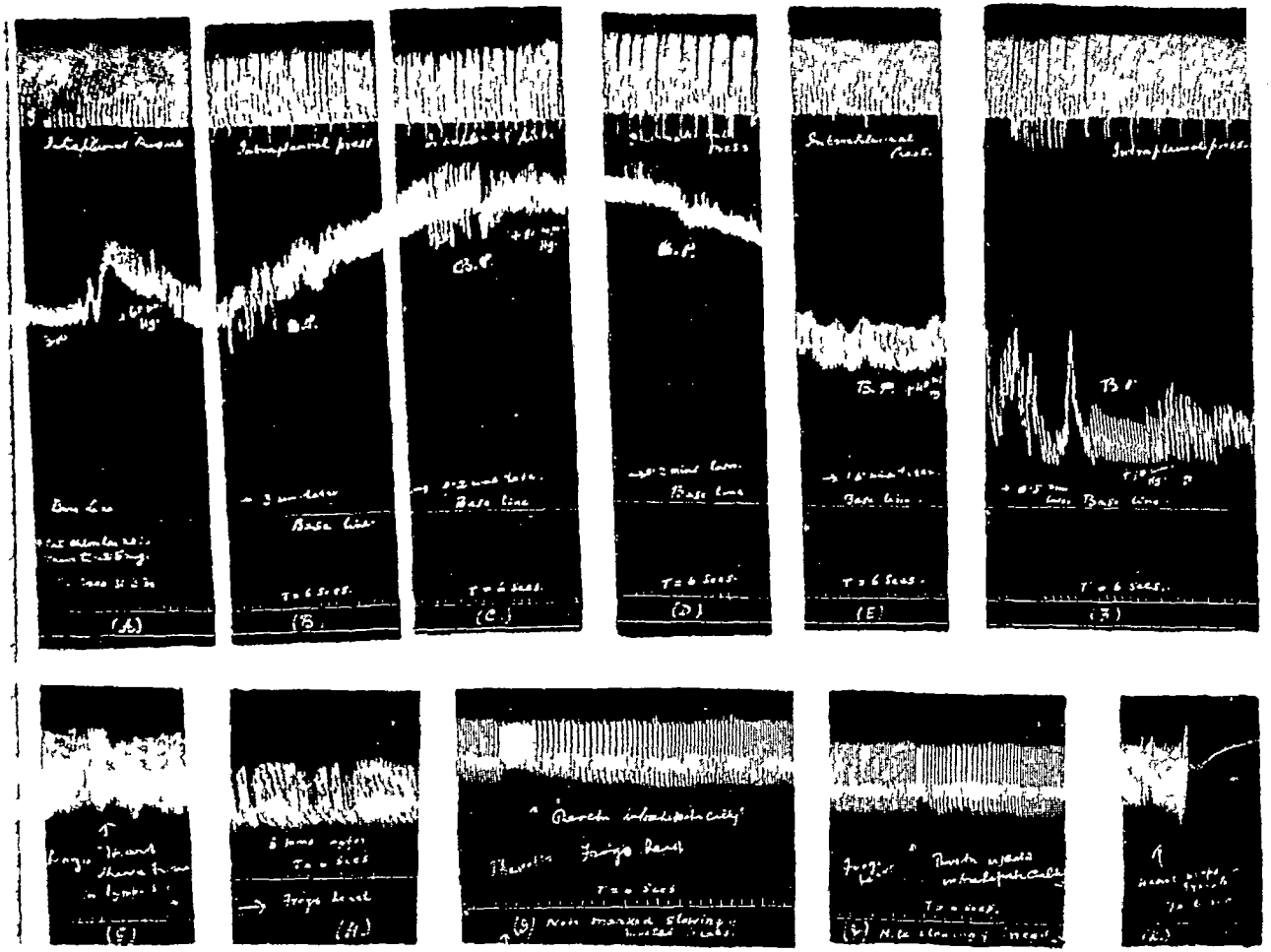
at the late stages are observed which are entirely due to circulatory disturbances (Graph I, *A, B, C, D and E*)

Effect on systemic blood-pressure—Injections of 0 1 to 0 25 mg of thevetin into the femoral vein of cats cause, within 20 to 30 seconds, a distinct and a maintained rise of the blood-pressure varying from 10 to 20 mm of mercury (Graph II, *A*, Graph III, *A* and *H*), a summation effect proportionate to the dose is sometimes obtained when another injection is given on the top of the first injection This is only the case when the dose does not exceed 0 5 mg Injection of larger doses, such as 1 mg to 5 mg, produces an irregularity in the blood-pressure curve in most animals (Graph I, *A, B, C, D, E and F*) Small irregular oscillations are at first produced followed by a well-marked rise in the blood-pressure curve If the dose does not reach the lethal limit this irregularity goes on for some time An effort on the part of the circulatory mechanism to re-adjust itself then becomes gradually evident and the blood-pressure shows a tendency to return to the normal level If, however, the lethal dose is pushed into the circulation quickly the irregularity in the blood-pressure curve is followed by a tremendous fall with subsequent collapse and death of the animal The rise in blood-pressure with small doses occurring in intact animals also occurs in decerebrated animals, though not to the same extent The pressor effect does not disappear when the sympathetic ganglia and nerve-endings are paralysed with nicotine and ergotoxine respectively This shows that the sympathetics play little or no part in the production of pressor effects When the vagal endings are paralysed with atropine, thereby cutting off all the inhibitory impulses, thevetin still causes a rise in pressure (Graph II, *E*) These findings lead us to conclude that the stimulation of the musculature of the heart or the vessel walls is perhaps responsible for the effect on the blood-pressure

Action on the heart—Frog's heart Thevetin was given intra-hepatically or in the lymph-sac in doses of 0 1 mg to pithed frogs and the movements of the heart were recorded on a slowly moving drum About 15 minutes after the injection a definite slowing of the heart and a diminution in the amplitude of the contractions are observed (Graph I, *G, H, I, J and K*) Sometimes the effects pass off completely in a short time, but if another dose is given at this stage the slowing becomes much more evident, the diastolic phase is shortened and the ventricle remains in a state of semi-contraction even during the phase of relaxation This stage of contraction slowly spreads over the whole chamber until the ventricle receives no more blood from the auricular systole, and eventually comes to a standstill The auricles may continue to beat for some time after the ventricle has stopped and later stop in a state of wide relaxation

Mammalian heart—Myocardiograph experiments On the mammalian heart thevetin has a stimulant action in small doses With a dose of 0 1 mg a very slight stimulation of the auricles is evident, but the ventricles are not affected at all (Graph II, *A*) With a higher dose, 0 25 to 0 5 mg injected intravenously, a distinct

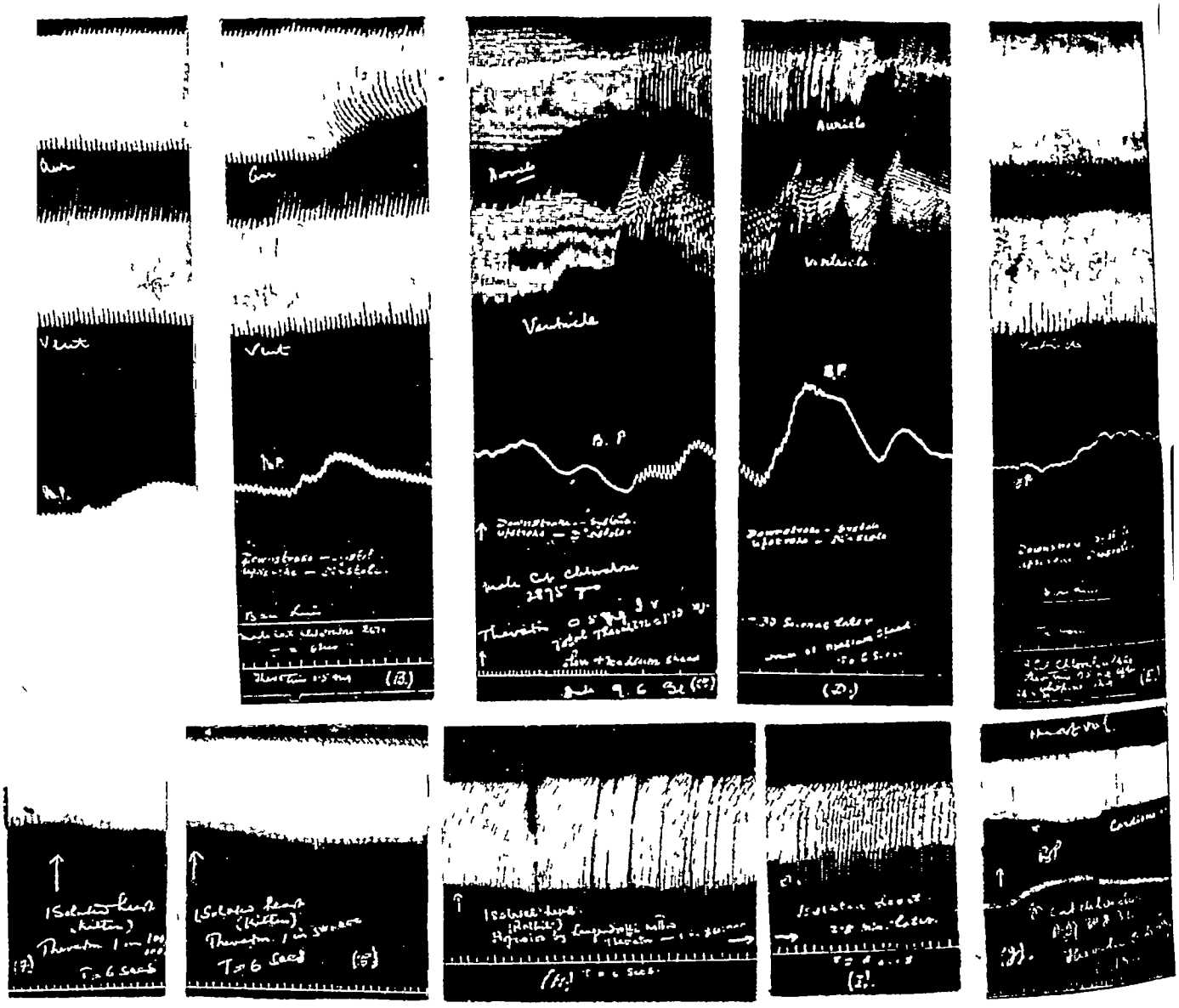
GRAPH 1



A, B, C, D, E and F —Record of blood pressure and intra pleural pressure in a cat under chloralose A toxic dose of 2 5 mg of thevetin injected in the femoral vein Note the rise in blood pressure after injection followed by marked irregularity in the blood pressure curve At *C*, 3 2 minutes later the blood pressure is at its highest level (80 mm of mercury from base line), and irregularities are well marked At *E* the blood pressure has come down to 40 mm of mercury (lower than the original level) but the rhythm seems to be fairly well maintained At *F*, the irregularity is very well marked Death follows soon after this stage

G, H, I, J and K —Frog's heart after injection of thevetin in gradually increasing doses in the lymph sac and intra hepatically The slowing of the heart becomes evident 6 minutes after injection, at this stage occasional missed beats are also to be seen Still later, regular missing of beats Final stoppage in systole

Graph II.



A, B, C, D and E —Myocardiograph tracings in cats under chloralose anæsthesia At *A*, with 0 1 mg of thevetin a very slight stimulation of the auricle is evident At *B*, with 0 5 mg of thevetin the weakening of the auricular systole is very well marked, the ventricle shows slight stimulation At *C* and *D*, with 1 25 mg of thevetin, the auricle shows marked inhibition both in its systolic and diastolic phases The ventricle follows suit a few seconds later Missing of beats and irregularities of conduction are also evident, at times, complete auricular paralysis is seen followed by slow, spontaneous ventricular rhythm The blood-pressure at these stages is very irregular *E* shows the myocardiographic record after atropine Stimulation of the auricle and ventricle is practically non existent

F, G, H, I and J —Isolated mammalian heart perfused by Langendorff's method and cardio-meter At *F* and *G* note stimulation of kitten's heart with dilutions of 1 in 100,000 and 1 in 50,000 At *H* and *I*, with a dilution of 1 in 10,000, irregularity is well seen in a rabbit's heart At *J*, with 0 25 mg thevetin stimulation, particularly in the systolic phase, is seen in the cardiometer tracing

slowing of the rhythm is evident In most cases a slight and transient depression of the auricular systole is noticed (Graph II, *B*) The ventricles do not show any signs of stimulation in the beginning, but later both the systole and diastole are slightly increased, the diastolic effect being more marked With bigger doses, such as 0 75 to 1 mg , the amplitude of contraction of the auricles is markedly diminished (Graph II, *C*) The ventricles in most cases show a diminution in the amplitude within 12 to 15 seconds of the auricular effect , gradually some inco-ordination in the passage of the impulses from the auricle to the ventricle becomes evident and the beats become irregular With still higher doses injected rapidly the auricular contractions show a rapid weakening in strength, fibrillary twitchings of the musculature and the rhythm is markedly slowed This gradually goes on till the musculature is paralysed so much so that the lever fails to record the beats *Fibrillation of the ventricle becomes evident at this stage, but it keeps on beating* for some time very irregularly until finally either the ventricle stops suddenly or a spontaneous and slow ventricular rhythm starts (Graph II, *D*) In order to determine whether the effect of thevetin was due to an action on the nervous mechanism or on the myocardium, the nervous connections of the heart with the central nervous system were severed In some experiments the brain was destroyed by passing a stout metal seeker into the cranium through the foramen magnum, while in others the vagi were severed at the level of the neck In both these series there was very little alteration in the effects produced by the glucoside, showing that the centre is not wholly responsible for the heart changes When the terminations of the vagi were paralysed by atropine the effect of the drug on the chambers of the heart disappeared to a great extent and the slowing of the chambers became much less evident (Graph II, *E*) It can be concluded, therefore, that stimulation of the inhibitory nervous mechanism is responsible partly for the slowing effect on the rhythm of the heart

The accelerator mechanism does not seem to play any part in the effect Paralysis of the sympathetic nerve-endings after large doses of ergotoxine does not alter the myocardiograph tracings in any way The blood-pressure is slightly lowered

Cardiometer experiments —Injection of 0 25 mg of thevetin into the femoral vein stimulates contractions The heart as a whole appears to be stimulated, both the systolic and diastolic phases being increased The output of blood is also definitely increased (Graph II, *J*)

Action on isolated mammalian heart —The isolated hearts of rabbits and kittens were perfused with oxygenated warm Locke's solution at pH 7 2 In dilutions of 1 in 100,000 to 1 in 50,000 thevetin produces a definite stimulation of the beats In weaker dilutions, e g , 1 in 200,000, no effect is noticed In stronger concentrations, e g , from 1 in 50,000 to 1 in 20,000, a definite increase in the force of the beats along with some slowing in the rhythm is observed With still stronger

concentrations, e g , 1 in 10,000 to 1 in 5,000, the muscles go into fibrillary twitchings and finally delirium cordis sets in, the heart ultimately stopping in diastole Paralysis of the vagal endings with atropine in such preparations, still produces the slowing effect but to a much smaller degree It appears likely that the drug has some direct action on the myocardium (Graph II, *F*, *G*, *H*, *I* and *J*)

Coronary outflow —In small concentrations such as 1 in 100,000 to 1 in 50,000, both the diastolic and the systolic phases become distinctly improved and the coronary outflow is slightly increased With higher concentrations, due to imperfect contractions of the ventricle, very little blood is pumped into the coronary arteries and the outflow is reduced to half or even less In one experiment, the coronary outflow decreased from 11 c c to 5 c c , half a minute after an injection of thevetin in dilution of 1 in 10,000

Pulmonary pressure —The pulmonary pressure shows a definite rise with 0 25 to 0 5 mg of thevetin This action is mainly cardiac due to stimulation of the musculature (Graph III, *E*)

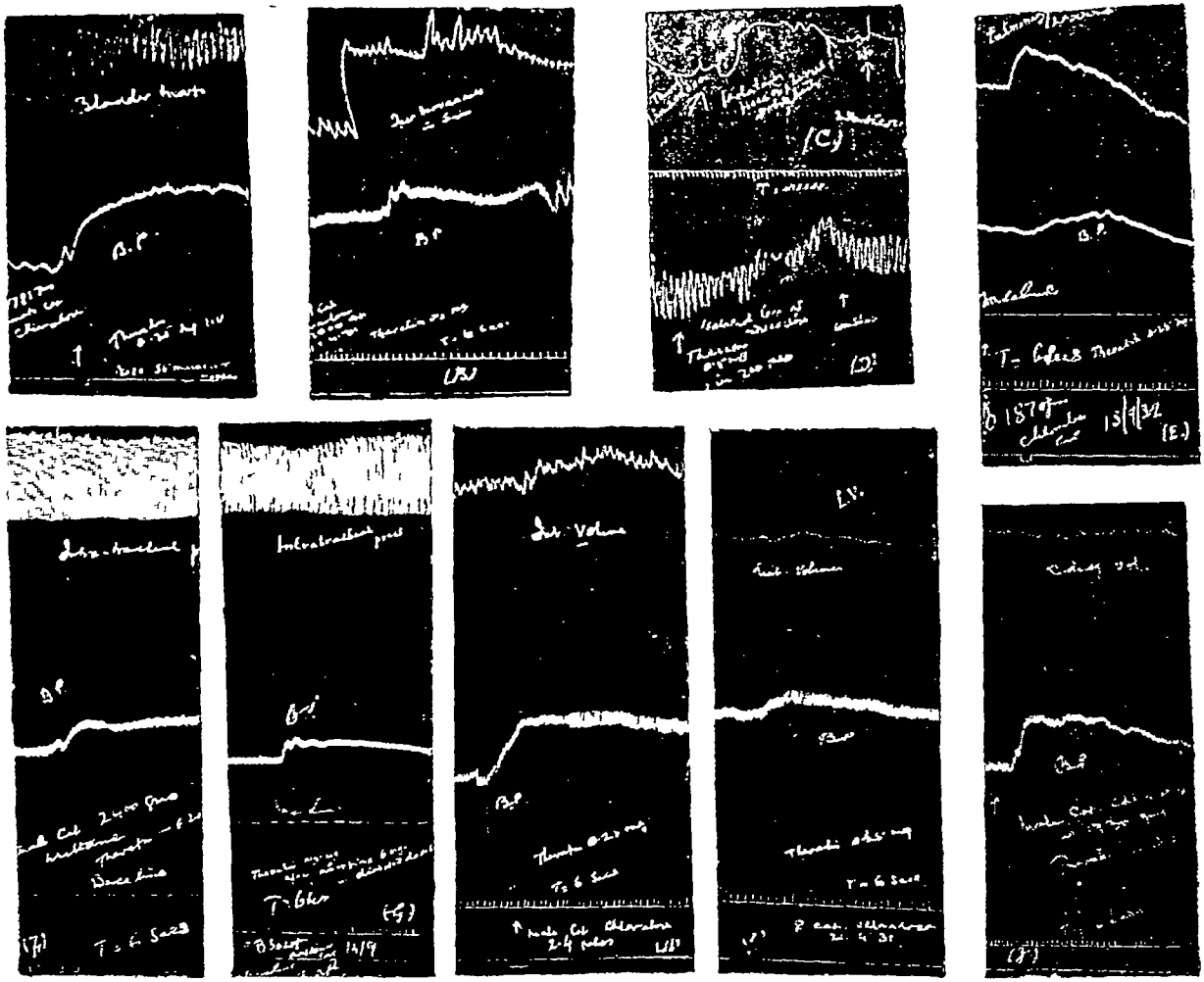
Action on the blood vessels —We have already seen that intravenous injections of thevetin in small doses produce a rise in the systemic blood-pressure This rise is still produced after the sympathetic ganglia are paralysed with nicotine and the sympathetic nerve endings are similarly treated with ergotoxine The rise is less in evidence after the vagal endings are paralysed with atropine All these suggest the possibility of the glucoside having some direct action on the musculature of the vessel wall

In order to determine any direct effect on the involuntary muscle of the blood vessels, a Trendelenburg preparation was put up After pithing a frog, a canula was introduced into the aortic arch and through this the vessels were perfused with Ringer's solution (frog) The outflow was noted by counting the drops that emerged from the cut-end of the inferior vena cava, sufficient time having been allowed for the preparation to give uniform results Thevetin was then added to the perfusate and the time taken for every 10 drops was recorded This shows a slight increase, showing that the alkaloid by its direct stimulant action on the musculature has produced constriction of the arterial system In warm-blooded animals such as the cat, addition of thevetin to the perfusate produces an appreciable constriction of the blood vessels of the artificially perfused hind limbs The constriction is still present, though to a much less extent, after the vasomotor nerve-endings were paralysed with large doses of ergotoxine The drug, therefore, must have a direct action on the involuntary muscle fibres of the vessel wall

Limb volume —The limb volume, as a rule, does not show any change after an intravenous injection of the drug In some cases a slight fall is observed, probably due to the mechanical effect of the rise in systemic blood-pressure (Graph III, *H*)

Intestinal volume and kidney volume —The effect on the blood vessels of the splanchnic area was studied by noting the changes produced on the volumes of such

Graph III.



A —Blood pressure and bladder movements with 0 25 mg of thevetin Note rise in blood pressure and increase of tone of the musculature of the bladder

B —Intestinal movements *in situ* shows definite stimulation with 0 5 mg of thevetin

C —Record of movement of an isolated piece of virgin rabbit's uterus in a Dale's bath at 37 5°C, and pH 7 4 Distinct stimulation of movements is to be seen after thevetin in concentrations of 1 in 400,000 to 1 in 200,000 are given The effect disappears to a great extent after washing

D —Isolated loop of intestine in Dale's bath Stimulation is evident in 1 in 200,000 dilution

E —Blood pressure and pulmonary pressure Temporary rise in pulmonary pressure is noticed with 0 25 mg of thevetin This is probably due to direct effect of the drug on the heart

F and *G* —Intratracheal pressure before and after atropine *F* shows no change and *G* shows slight slowing of the rate and relaxation of the bronchioles

H, *I* and *J* —Intestinal volume, limb volume and kidney volume with thevetin 0 25 to 0 5 mg Intestinal volume shows a slight rise with small doses while limb volume and kidney volume show practically no change

organs as the spleen, kidney and the intestines The intestinal volumes show a slight increase (Graph III, *G*), the kidney volume in most cases maintains a normal level (Graph III, *I*) Though one would expect a constriction of the volumes of these organs due to the effect of the drug on the vessel wall, this effect appears to be counter-balanced in most cases by the general rise in the systemic pressure and the volume changes usually keep pace with the systemic blood-pressure

Action on uterus and plain muscles—Thevetin in 0 25 mg doses stimulates the plain muscles, as evidenced by the increase in tone and movements of the urinary bladder (Graph III, *A*) The non-pregnant uterus of rabbits is markedly stimulated by such dilutions as 1 in 200,000 to 1 in 100,000 (Graph III, *C*) This is probably due to direct action of the drug on the smooth muscles of the bladder and uterus

Discussion—From the experimental data it would appear that the effect of thevetin is specially marked on the heart and the circulatory system In very small doses it has got a distinct tonic action on the heart and circulation The blood-pressure is raised and is maintained at a high level for a fairly long time The pulse is slowed This pressor effect is partly due to increased cardiac output and partly to the stimulant effect of the drug on the plain muscles of the blood vessels In cardiometer experiments there is distinct evidence of the stimulant effect on the heart In myocardiograph experiments both the systolic and diastolic phases are found to be more complete This effect is partly due to stimulation of the vagal endings and partly due to direct effect of the drug on the cardiac musculature Stimulation of the vagal endings should produce a slowing and depression of the auricles as well as of the ventricles But in most of the myocardiograph experiments the auricles are affected much more than the ventricles This result is difficult to interpret but the possible explanation might be that, although both the factors are operating in each case, in the case of the auricles the nervous factor predominates, while in case of the ventricles the stimulant effect of the drug on the musculature overbalances the nervous effect This is in accordance with the findings of Starling who has pointed out that it is doubtful if the vagus has any direct action on the mammalian ventricle, its effect at any rate is slight as compared with that on the venous end of the heart In large doses thevetin produces a marked inhibition of the auricles and the ventricles The rate, rhythm, and the force of the beats are all markedly decreased Conduction of the impulses seems also to be affected and marked irregularity in the auricular and ventricular contractions are evident In this stage it would appear that an excess of inhibition overshadows for the time being the muscular effect, the heart-beat is very irregular and slow but, provided the effect is not too strong, the output at each systole is still quite enough for the circulation to be kept up temporarily and the systemic blood-pressure is actually higher than normal in some animals Still larger doses of thevetin, it has

been shown, produce fibrillary twitchings of the auricular muscle and sometimes complete paralysis This result is probably due entirely to muscular action as this effect cannot be abolished by paralysing the vagal endings with atropine The quickening of the heart, noticed in some cases during the last stages after a big dose of the drug, is probably due to an action on the excito-motor area It is very difficult to say at this stage whether the irregularity of conduction noticed in some cases is due to any special action of the drug on the nodal tissue and the Bundle of His, like some of the digitalis groups of drugs For elucidation of this point further investigations are required preferably with the electrocardiograph The determination of the P-R interval before and after the drug is likely to be of great use

Summary and conclusions

1 One of the active principles of *Thevetia nerifolia* (yellow oleander) is a crystalline glucoside—thevetin

2 Thevetin is very toxic to frogs, mice, guinea-pigs, cats and higher animals It has no irritant action locally on the conjunctiva or on the skin or when injected subcutaneously

3 The glucoside has no action on the digestive enzymes It has little or no direct effect on the respiration

4 Thevetin has a direct stimulant action on the plain muscles of the intestine, bladder, virgin uterus and blood vessels

5 Thevetin has a pronounced effect on the circulatory system, which resembles in many respects the drugs belonging to the digitalis group

6 This action appears to be due partly to stimulation of the vagal mechanism and partly to direct stimulation of the cardiac musculature The action manifests itself either on the nervous or on the muscular elements of the heart according as a small or a large dose is administered It is not possible to state whether conduction of the impulses from auricle to ventricle through the Bundle of His is affected

7 The glucoside, on account of its cardiotonic properties, should be a potent therapeutic agent, but the margin between the therapeutic and toxic limit seems to be too low to warrant its safe administration

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ON RABIES COMPLEMENT FIXATION IN RABIES THE TECHNIQUE, ITS PURPOSE AND ASSOCIATED CONSIDERATIONS

BY

MAJOR S D S GREVAL, B SC (Punjab), M D, CH B, D P H (Liverpool), I M S,

Officiating Director, Pasteur Institute of India, Kasauli

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I THE TECHNIQUE

1 *The reagents*

(*i*) The hæmolytic system as prepared for a Wassermann reaction and used in the same proportions For the titration of the complement Method No 4 of the Medical Research Council (1928) has been followed

(*ii*) Saline as used in a Wassermann reaction

(*iii*) The antigen This is a filtrate of a 3 months' old 5 per cent carbolized Paris (virus) sheep (brain) vaccine of the Pasteur Institute, Kasauli A 10 c c ampoule is shaken up, poured out into a funnel provided with a filter-paper and left to filter for 3 hours The almost clear filtrate is made homogeneous by shaking before use

The vaccine is prepared thus The brain of a sheep is made into an 8 per cent suspension with 1 per cent carbolic acid in normal saline, this is incubated for 24 hours at 37°C and then diluted to a 5 per cent suspension with normal saline, this is kept at room temperature for about 30 days before use

(*iv*) The serum, 1 in 5, from a sheep immunized against dead fixed Paris virus of rabies

2 *The ensemble*

To suit specially a small volume procedure, plasticine stands, small quill tubes with stirrers, pipettes with teats and a water-bath with glass sides After descriptions by Hewlett (1926) and Brown (1915, 1918)

(*a*) Left at room temperature for 1 hour (*b*) Left in water-bath at 37°C for 45 minutes (*c*) Left at room temperature again for 15 minutes (*d*) Sensitized red cell suspension, 1 vol, added to all the tubes (*e*) Left in water-bath for half an hour (*f*) Lysis and inhibition of lysis noted (*g*) Left in the cold overnight (*h*) Traces of lysis noted

Reagents	Serum control Tubes —		Antigen control Tubes —		Test proper Tubes —		
	1st	2nd	1st	2nd	1st	2nd	3rd
Complement, M H D in 1 vol	2	3	2	3	8	10	12
Serum dilution	1 vol		*Nil*		1 vol		
Antigen	*Nil*		1 vol		1 vol		
Saline	1 vol		1 vol		*Nil*		

For weak sera, smaller doses of the complement are used in the test proper, e g , 3, 4 and 5 M H D or 4, 5 and 6 M H D or 6, 7 and 8 M H D instead of 8, 10 and 12 M H D To detect the merest trace of the immune body in a very weak serum the dilution of the serum is lowered, e g , a 1 in 4 or 1 in 3 dilution is used instead of a 1 in 5 dilution

For strong sera the dilution of the serum can be raised from 1 in 5 to 1 in 7, 1 in 10 or even higher The dose of the complement need not be raised above 12

A large number of repetitions during the last six months have shown that constant and comparable results can be obtained by keeping to the above figures

For human sera, from cases treated for serious bites, a filtrate from a normal sheep brain (a ' blank ') is also used as an extra control, in addition to the antigen, to guard against a non-specific reaction against the sheep brain The results obtained so far have not been conclusive Modifications of the technique for this purpose are on trial

3 *Reading of results*

M H D of the complement in the fully lysed serum control *plus* the M H D in the fully lysed antigen control *minus* 1 *equals* the M H D. needed

for a complete lysis when the serum and the antigen are put together (both being anti-complementary) Any amount needed for an incipient lysis above this figure *plus* 1 represents the complement fixation measured in M H D

The reason for subtracting 1 is this Had the two controls (the antigen control and the serum control) been mixed together as they stood in the tubes, there would have been two volumes of the red cell suspension to lyse, while in the test proper there is only one volume of the red cell suspension to lyse

The reason for adding 1 is this The amount of complement needed for a complete lysis would, of course, produce a complete lysis in the tube containing the amount, when there is, with this amount, an inhibition of lysis instead, or only an incipient lysis which amounts practically to an inhibition, 1 M H D has already been fixed, this 1 M H D must be added to the total above the figure needed for the incipient lysis

This mode of calculation is adopted as a working rule only A negative serum which is not abnormally anti-complementary reduces the anti-complementary titre of the antigen in reality

4 *Typical examples*

Cases	Serum control Tubes —		Antigen control Tubes —		Test proper Tubes —		
	1st (2 doses)	2nd (3 doses)	1st (2 doses)	2nd (3 doses)	1st (8 doses)	2nd (10 doses)	3rd (12 doses)
A	—	—	—	—	+	+	+
B	—	—	—	—	+	+	+(?)
C	—	—	—	—	+	+	—
D	—	—	—	—	+	±	±
E	—	—	—	—	—	—	—
F	±	—	±	—	+	+	+
G	+	+	+	+	+	+	+

CALCULATIONS

Case A —

M H D fixed = 12—(2 + 2—1) + 1=10 *at least* (the end point not reached)

Case B —

M H D fixed = 12—(2 + 2—1) + 1=10 '?' indicates a trace of lysis (an incipient lysis) and a reaching of the end point

Case C —

M H D fixed = 10—(2 + 2—1) + 1=8 or over, but not over 9

Case D —

'Irregular' reaction observed

M H D fixed regularly = under 10—(2 + 2—1) + 1 = under 8

M H D fixed irregularly =12—(2 + 2—1) + 1=10 at least

Case E —

Negative

Case F —

M H D fixed = 12—(3 + 3—1) + 1=8 *at least*

Case G —

Results cannot be read Should be repeated with higher doses in the controls

A remark may here be made on the 'irregular' reactions They occur contrary to arithmetical considerations In case D, a ± reaction is obtained with 10 M H D of the complement This lack of complete lysis indicates a lack of only a fraction of an M H D With 12 M H D there should not be any inhibition of the lysis at all Hence the 'irregularity' Such reactions, apparently unrecognized by other workers in complement fixation, are of quite frequent occurrence I have drawn attention to them previously in this *Journal* (Greval *et al*, 1930)

5 *Non-specific controls*

Combinations of (*a*) normal sera and a filtrate as described above and of (*b*) immune sera and a filtrate prepared from normal brains (a 'blank') have given negative results with the lowest doses of the complement A bacterial anti-serum from sheep (anti-plague) has also given negative results

II THE PURPOSE

The immediate purpose of the technique is to determine by a serological method the potency of the anti-rabic sera employed in the immunization experiments which are being conducted on animals, at present, at the Pasteur Institute, Kasauli The

usual method of estimating the potency of such sera by a neutralization of the virus *in vitro*, as shown by the escape of an animal inoculated with a mixture of the virus and the serum, besides being a time-consuming process is not capable of giving exactly comparable results As a parallel procedure and an occasional check, the actual protection afforded by the serum can be tested from time to time by animal inoculation

Another purpose in view is to compare, serologically, the efficacy of the various anti-rabic vaccines, by testing them against a constant serum This comparison will also provide a means of differentiating the various 'strains' of rabies virus, an inquiry recommended by the International Rabies Conference of Paris (League of Nations, 1927)

Yet another purpose is to test each attending case at real risk, by a suitable modification of the technique, for evidence of immunity, at a suitable stage, with a view to prolonging or modifying the treatment, if necessary

The ultimate objective is to discover the means, by eliminating consumption of time and lack of comparability, of producing a potent anti-rabic serum which may be used as an adjunct in vaccine therapy in late and badly bitten cases and even as a cure in a declared case of hydrophobia as anticipated by King (1932)

III Associated considerations

1 *The anti-rabic serum*

Semple (1903, 1904, 1911) obtained an anti-rabic serum from ponies after long periods of immunization by subcutaneous route This serum neutralized a fixed rabies virus *in vitro* but was without any appreciable effect *in vivo*

A serum obtained from sheep, after a period of immunization extending over eight months, by subcutaneous route, has recently been found to neutralize a street virus (1st passage) after 12 hours' contact at room temperature The titre of the serum as ascertained by a complement fixation reaction was very low It fixed 'irregularly' 3 M H D of the complement only

By adopting the intravenous route a serum of a much higher titre was obtained in 2 months It fixed 10 M H D of the complement 'regularly' The technique described in this paper was developed with this serum The 'irregular' reaction still persisting towards the end of the titre is, in my experience, indicative of a possibility of obtaining a still higher titre Besides, the maximum intravenous dose has not yet been reached

The sheep anti-rabic serum contains an immune body which is not affected by a temperature of 55°C for 30 minutes Some specimens of it, as a matter of fact, were definitely improved by heating at 55°C for 10 minutes This improvement

was not dependent on the destruction of the native complement as the serum was over two weeks old

An anti-rabic serum obtained from buffaloes, after a period of immunization extending over six months, by subcutaneous route, was not found to be as good It fixed 'irregularly' 6 M H D of complement Heating for 10 minutes at 55°C completely destroyed its immune body Exactly similar results were obtained by me with the anti-plague serum from sheep and buffaloes, last year, at the Haffkine Institute, Bombay

2 *The antigen*

In the early part of this work filtrates from several freshly made vaccines, from undried nervous matter, were employed as antigens and all gave poor reactions A filtrate from a carbolized 'ether' vaccine being the safest to handle and easiest to keep was used the most It appeared to give a better reaction than every other vaccine until the factor of age was considered and controlled Then the fact emerged that a vaccine as an antigen in a complement fixation reaction improved with age It was then decided to use only a three months old vaccine which incidently is discarded as time-expired according to the custom of the anti-rabic clinics in India

Filtrates from fresh vaccines (under a week old) give very poor reactions In all the samples so far tested the reaction has improved with the age of the vaccine A filtrate from a three weeks old vaccine gives as good a reaction as the filtrate from a three months old vaccine The upper limit of the reaction has not yet been determined

Rabbit-brain vaccines have been found to give much poorer reactions than sheep-brain vaccines

The deposit-free fluid from a vaccine appears to be possessed of the same antigenic power whether it is removed by decantation, filtration or centrifugalization Its degree of opacity varies Its anti-complementary power again is the same A filtrate from an L3 Candle is very poor in antigen

A washed deposit fails to fix any complement regardless of the fact whether it is obtained from a freshly made vaccine or from a three months old vaccine It is not more anti-complementary than the deposit-free fluid and it does not interfere with the reading of the results The red cells in a mixture containing a resuspended deposit fall to the bottom of the tube much earlier than the deposit A positive reaction, if one occurred with the deposit, could not be missed

A vaccine made according to Hindle's (1929) process of making a vaccine for yellow fever was found to be very anti-complementary As a fresh vaccine it did not fix any complement at all

The antigen recommended by Marie and Urbain (1929) was not tried nor was their technique For reasons of simplicity and safety of the antigen and familiarity with the British technique the antigen and the technique described were preferred

3 *The scope of serological methods in determining immunity*

With regard to the active immunity induced in a subject, according to Topley and Wilson (1929) 'there is no doubt at all, from the general experience of immunological workers that the correlation between active immunization and the appearance of sensitizing anti-bodies in the blood is a high one Again, 'in this connection we may refer to Glenny's studies in anti-toxic immunity, since the principles involved are clearly applicable to immunity in general' * * * * * * Thus the immunized animal differs from its non-immune fellow, not only, nor chiefly, in the possession of circulating anti-toxin, but in an altered reactivity of the anti-body-producing mechanism, in virtue of which it responds promptly and effectively to further stimuli' The lack of correlation between serological reaction and actual protection, when the latter exists without the former, can thus be explained

With regard to the correlation between the anti-body titre of a particular sample of serum, as determined by serological methods, and its power to protect the situation is different The correlation, if any, must be established by a protection experiment Even a correlation between the titre and a definite neutralizing effect *in vitro* may be very significant A difference in action *in vitro* and *in vivo* may only be due to a difference in concentration

SUMMARY

1 A technique of complement fixation with an anti-rabic sheep serum has been described in details

2 Preliminary observations on the immune body and the antigen have been made

3 Comments on the scope of serological methods in determining immunity have been made

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FILARIASIS IN TRIVANDRUM

BY

M O T IYENGAR, B A , F Z S ,

Medical Entomologist, Travancore

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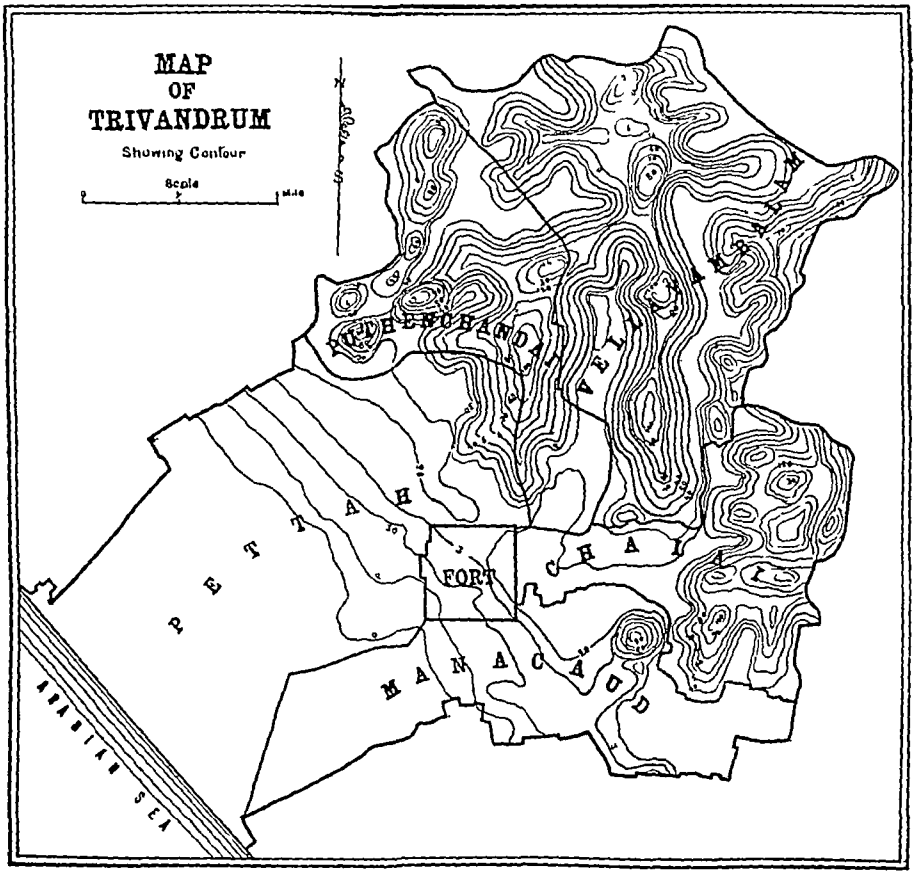
AN investigation into the incidence of filariasis in Trivandrum (South India) has recently been concluded and the findings are briefly reported in the present article

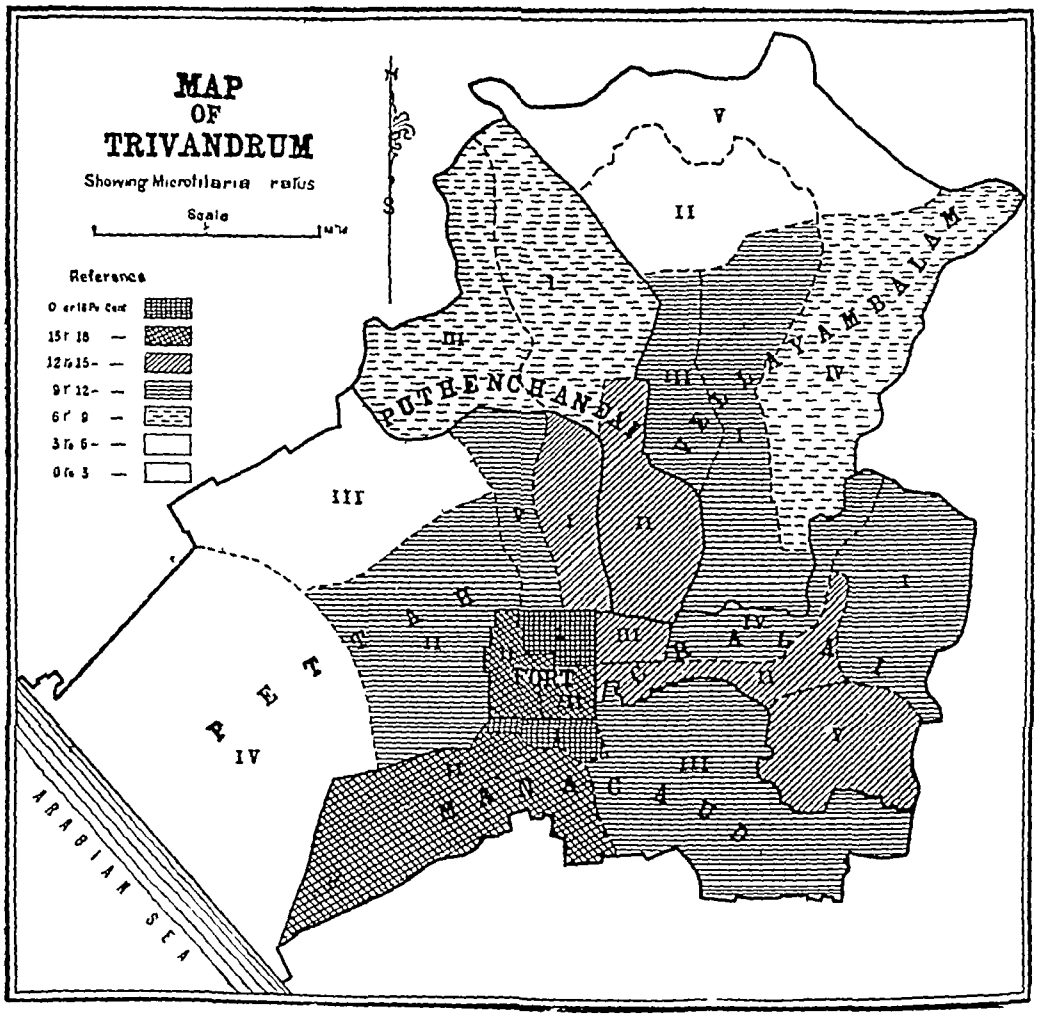
MAP 1



Trivandrum, the capital city of Travancore State, is situated on the western sea-coast of India and covers an area of 11 square miles A large part of the area

is undulating, dotted over with hillocks and traversed by valleys The area could be roughly divided into two regions, an elevated and undulating region on the north and east, and a flat low-lying one on the south and west, as can be seen from the contour map of Trivandrum here reproduced (Map 1) The general fall of land is from the north-east to the south-west

MAP 2



Trivandrum is divided into six municipal wards Fort and Manacaud are two wards on the south , Chalai ward is situated on the east, Puthenchandai and Vellayambalam on the north, and Pettah on the west Each of the six wards is again divided into smaller sections Fort, Manacaud and Puthenchandai wards are split up into 3 sections each, while the remaining three wards are divided each into five sections Map 2 shows the 24 sections into which the municipal area is

divided Trivandrum has a population of 96,000 Fort ward and the areas immediately adjoining it constitute the most densely populated areas

To determine the incidence of filarial infection and of filarial disease in the different sections of Trivandrum, blood films (thick films) were taken from the resident population of Trivandrum and any manifestations or history of filarial disease were noted at the same time The films were taken at night between 9-30 P M and 12-30 A M and were stained and examined for microfilariæ on the following day A record was maintained of the results Over 31 000 persons were examined in this series and the results have been analysed to bring out the variations in the different sections of the town in regard to (*a*) incidence of filarial infection as evidenced by the presence of microfilariæ in peripheral blood at night, (*b*) incidence of filarial disease such as lymphangitis and elephantiasis, and (*c*) *filarial endemicity* The filarial endemicity of an area is based on a combination of the two data, the microfilaria rate and the filarial disease rate, as either of these by itself would not give a reliable idea of the endemicity The incidence of infection with *Wuchereria bancrofti* (*a*) does not entirely represent filarial endemicity as it excludes persons who, as a result of the onset of filariasis, have become negative for microfilariæ The filarial morbidity rate (*b*) is also not a correct measure as it excludes persons already infected with *W bancrofti* who are likely to develop filariasis at a subsequent date A combination of the two factors—incidence of filarial infection and incidence of filarial disease—thus represents a more accurate measure of filarial endemicity

Table I furnishes the details in regard to (*a*) the microfilaria rate, (*b*) filarial disease rate, and (*c*) the filarial endemicity figures, of the 24 sections of Trivandrum —

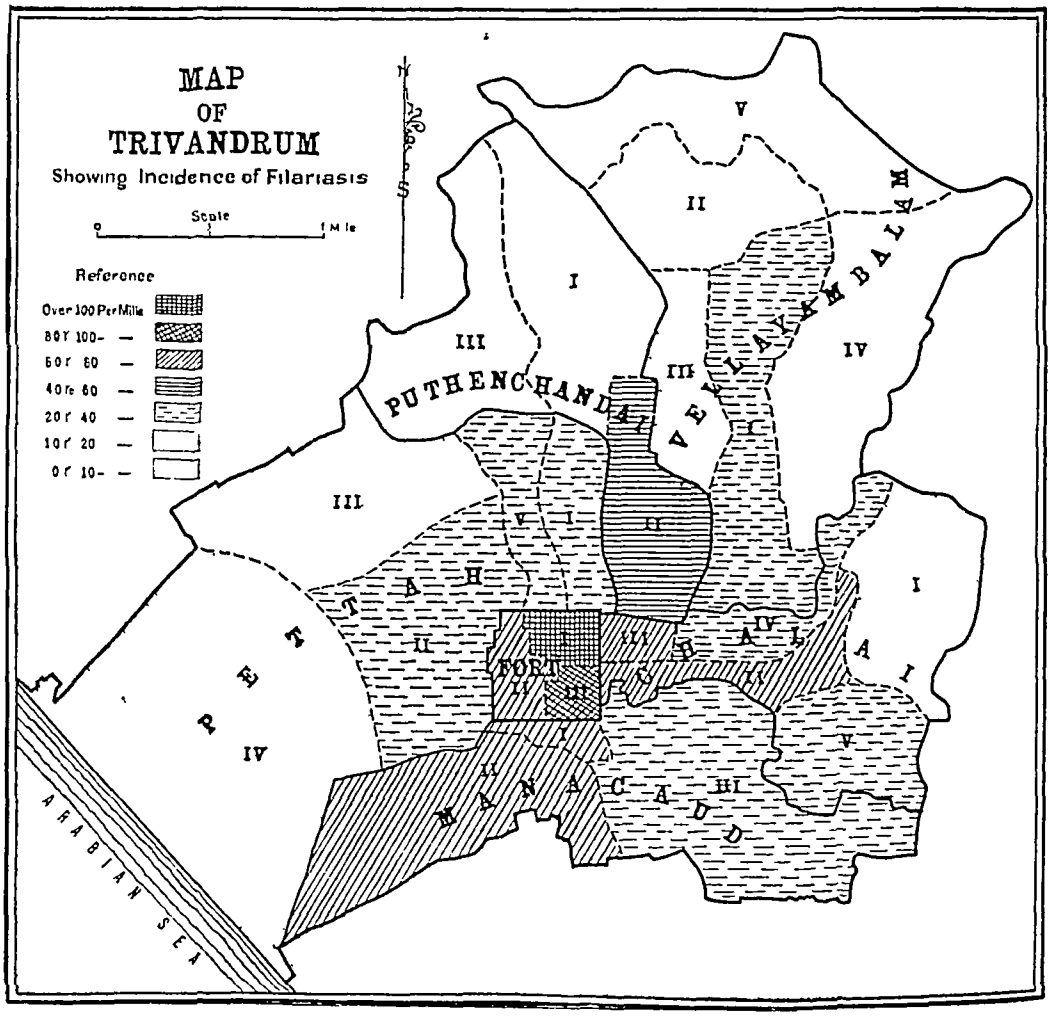
TABLE I

Sections	Total number examined	Number positive for microfilariæ	Microfilaria rate per cent	Number with symptoms of filariasis	Filariasis rate per mille	Number positive for microfilariæ or for filarial disease or both	Filarial endemicity rate per cent
1 Fort I	524	96	18 3	82	156 5	171	32 6
2 „ II	822	134	16 3	58	70 6	190	23 1
3 „ III	558	98	17 6	53	95 0	146	26 2
4 Manacaud I	1,435	289	20 1	104	72 5	380	26 5
5 „ II	416	63	15 1	30	72 1	92	22 1
6 „ III	1,335	146	10 9	50	37 5	195	14 6

The chances of finding microfilariæ in filarial subjects are comparatively greater in the early stages of filarial affections, such as mild lymphangitis cases, and as the duration of the disease is longer and the affections become more chronic, there is a decreasing incidence of microfilariæ in the blood Among cases of elephantiasis of the limbs, the microfilaria rate was only 3 3 per cent

The data in regard to the incidence of filarial disease in the different sections of Trivandrum are furnished in Table I Map 3 shows the variations in the intensity

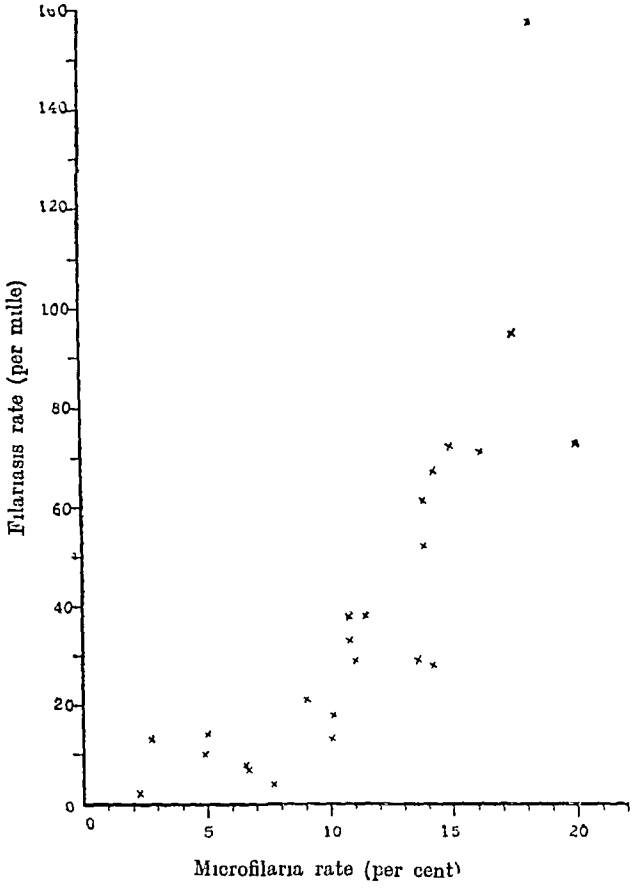
MAP 3



of filarial affections in the 24 sections of Trivandrum Areas with a heavy incidence of filariasis are Fort I, Fort III, Manacaud I, Manacaud II and Fort II with filariasis rates of 156 5, 95 0, 72 5, 72 1, and 70 6 per mille respectively T e least affected areas are Vellayambalam IV and V and Puthenchandai I and th filarial disease rates lower than 8 per mille

The distribution of filariasis in Trivandrum corresponds closely with the distribution of filarial infection A comparison of Map 2 with Map 3 brings out the close relation between the microfilaria rate and the filarial disease rate Areas with heavy infections have a high incidence of filarial disease, and vice versa There is a high degree of positive correlation between the microfilaria rate and the filariasis rate which is represented graphically in Chart 1 More accurately this correlation is expressed by the formula, $r = + 0\ 8459 \pm 0\ 0392$

CHART 1

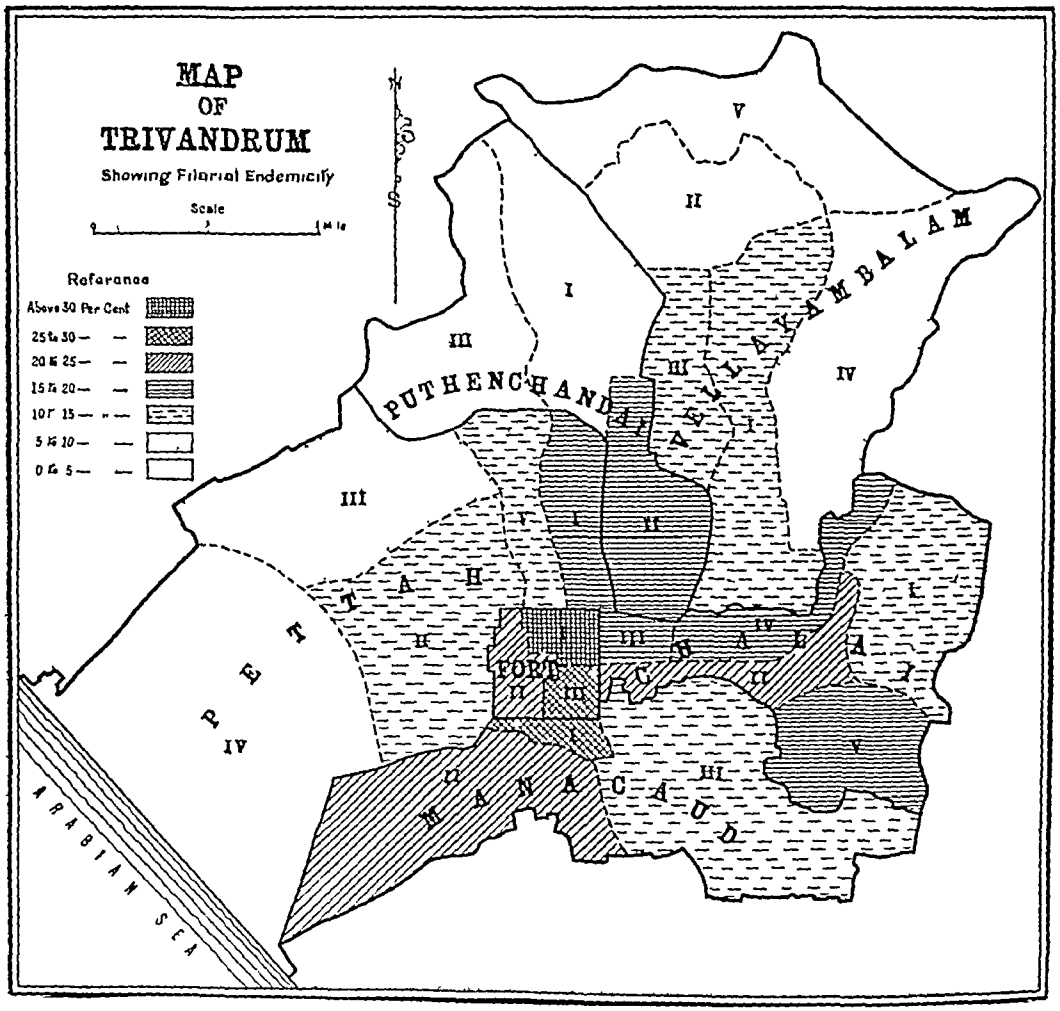


(c) *Filarial endemicity*

The filarial endemicity figure, which is based on a combination of the microfilaria rate and the filarial manifestation rate, represents the incidence of persons positive for microfilariæ or for filarial diseases or for both The endemicity figures

of the different sections of Trivandrum are furnished in the last column of Table I The distribution of filarial endemicity in the area is represented on Map 4 The central area has an endemic value of 25 per cent and over , in the outlying areas the endemic figure is lower than 4 per cent

Map 4



Intensity of infection

During the course of the examination of thick films for microfilariæ, the number of microfilariæ observed in positive films was also recorded In working out the average number of microfilariæ per positive film for each of the different sections of Trivandrum, some marked differences were observed in the average intensity of filarial infection Admittedly there is the possibility of error in these figures due to variations in the quantity of blood taken in making the thick smears But,

although, the quantity of blood taken was not measured by pipettes, it was roughly standardized so that the film consisted of an approximate size and thickness In view of the very large number of blood films taken in the same manner by the same persons, it is presumed that in working over a large series consisting in this instance of over 31,000 specimens, the error due to small variations in the quantity of blood taken for the thick films may not be considerable With this admission let us discuss the results

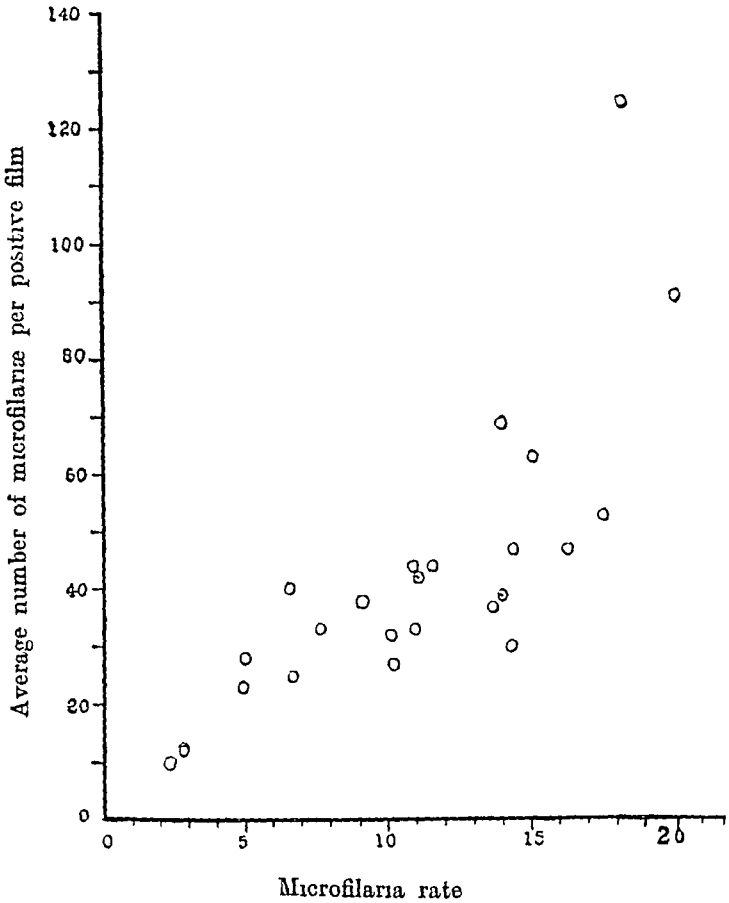
The respective microfilaria rates (per cent) and the intensity of infestation (average number of microfilariæ per positive film) of the 24 sections of Trivandrum are furnished in Table II below —

TABLE II

Serial number	Section	Incidence of filarial infection (microfilaria rate per cent)	Intensity of infestation (average number of microfilariæ per positive film)
1	Fort I	18 3	124 0
2	„ II	16 3	46 7
3	„ III	17 6	52 6
4	Manacaud I	20 1	90 7
5	„ II	15 1	62 8
6	„ III	10 9	33 4
7	Chalai I	10 2	26 6
8	„ II	14 4	46 9
9	„ III	14 0	68 6
10	„ IV	11 6	43 7
11	„ V	14 3	29 7
12	Puthenchandai I	6 7	25 1
13	„ II	14 0	39 0
14	„ III	7 7	32 6
15	Pettah I	13 7	37 2
16	„ II	10 9	43 5
17	„ III	5 0	27 6
18	„ IV	2 8	12 4
19	„ V	11 1	42 3
20	Vellayambalam I	9 1	38 4
21	„ II	4 9	22 6
22	„ III	10 1	31 5
23	„ IV	6 6	39 9
24	„ V	2 3	9 9

The correlation between the two observations, namely microfilaria rate and average infestation, is represented graphically in Chart 2 One observes here a fair degree of positive correlation, the value of which is expressed by the formula, $r = + 0.7362 \pm 0.0645$

CHART 2



EARLY APPEARANCE OF FILARIAL INFECTION AND OF FILARIASIS

In this series of observations, microfilariæ have been observed in the peripheral blood in several instances at very early ages The earliest age at which microfilariæ were observed was that of a child aged 2 years and 3 months with a count of 187 microfilariæ in thick film Another instance has been recorded of a child (2 years 4 months) with a microfilaria count of 3 But generally, it is uncommon to find positive cases below 4 years of age From the 4th year onwards, positive instances are less uncommon In the heavily infected areas of Trivandrum children of six years and above have an infection rate higher than 10 per cent.

In regard to filariasis, clinical symptoms of filariasis were observed in a single instance as early as the 8th year, in the case of a boy who had periodic attacks of lymphangitis and whose blood was positive for microfilariæ Definite symptoms of elephantiasis were observed in the case of a boy aged 11 years who had elephantiasis of both legs From the 14th year and onwards, elephantiasis was observed more commonly Several boys and girls aged 14 had elephantiasis of the leg and one boy aged 16 was observed to have a scrotal affection besides elephantiasis of the leg

Incidence of filarial infection and of filariasis in relation to age

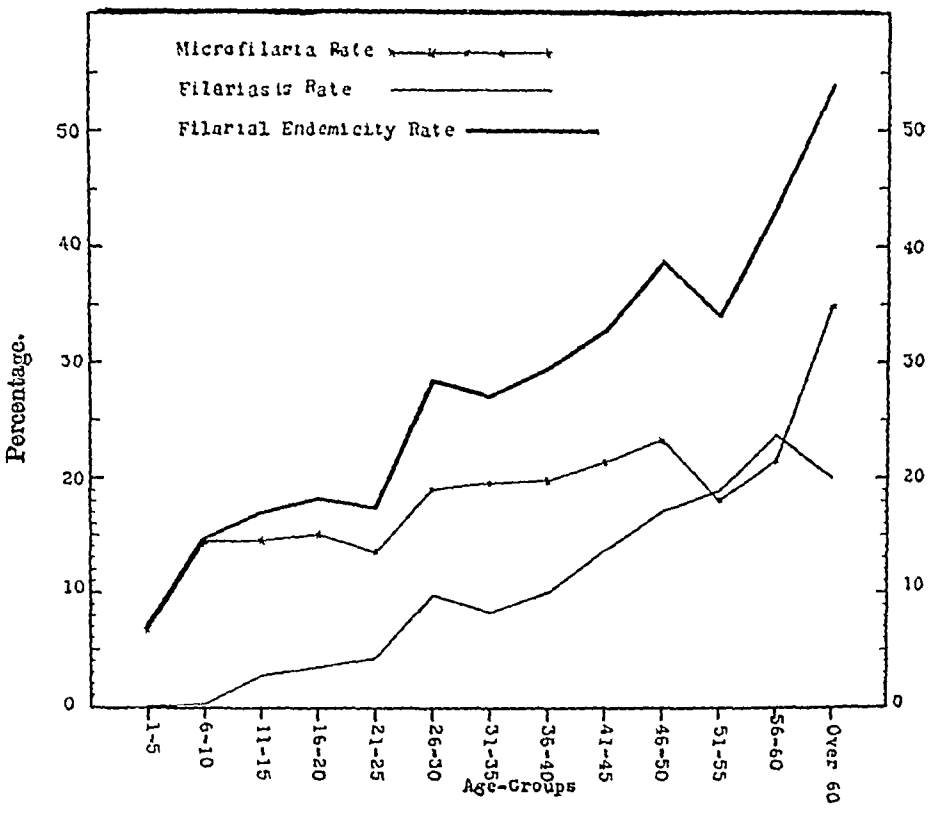
The records of five of the more severely affected sections of Trivandrum (e g , Fort I, II, III, and Manacaud I and II) have been analysed to enable a comparative study of the incidence of filarial infection and of filariasis at different ages among the population of an endemic area The results are furnished in Table III —

Table III

Serial numbers	Age group	Total examined	Number positive for micro filariæ	Per centage	Number positive for filariasis	Per centage	Number positive for micro filariæ or for filariasis or both	Per centage
1	1 to 5	74	5	6 8	0	0 0	5	6 8
2	6 „ 10	303	44	14 5	1	0 3	44	14 5
3	11 „ 15	391	57	14 6	11	2 8	66	16 9
4	16 „ 20	443	66	14 9	15	3 4	81	18 3
5	21 „ 25	470	63	13 4	20	4 3	81	17 2
6	26 „ 30	410	78	19 0	40	9 8	117	28 5
7	31 „ 35	362	71	19 6	30	8 3	98	27 1
8	36 „ 40	349	69	19 8	35	10 0	103	29 5
9	41 „ 45	277	59	21 3	38	13 7	91	32 8
10	46 „ 50	245	57	23 3	42	17 1	95	38 8
11	51 „ 55	182	33	18 1	34	18 7	62	34 1
12	56 „ 60	148	32	21 6	35	23 7	64	43 2
13	Over 60	135	47	34 8	27	20 0	73	54 1

These results are represented graphically in Chart 3 —

CHART 3



The incidence of filarial infection is comparatively low (6 8 per cent) in the first age group 1 to 5 years It rises to 14 5 per cent in the next age group (6 to 10 years) and remains practically at the same level during the succeeding three age groups In the 26 to 30 year group it rises to 19 0 per cent and from that point to the 56 to 60 year group it maintains nearly the same level In the last age group (61 years and above) it rises to 35 per cent

The incidence of filariasis in the different age groups is shown by a graph on the above chart (Chart 3) In the first two age groups the incidence of filariasis is practically *nil* In the 11 to 15 year group it is 2 8 per cent and it rises steadily in every succeeding age group and reaches a high level (20 per cent and over) in the last two groups

The filarial endemicity figures of the different age groups are based on the percentage incidence of persons that are positive for microfilariæ or positive for filariasis or for both This represents both the early stages in the infection in which clinical symptoms of filariasis are not apparent, as well as the later stages in which definite indications of filariasis occur This curve shows a steady increase as the

age advances and demonstrates clearly that the incidence of filarial endemicity is directly proportional to age and that it rises with the increase in the period of exposure to infection

These observations show that both filarial endemicity and the filariasis rate exhibit a steady increase in the increasing age groups, they vary directly with the period of exposure to infection On the other hand, the microfilaria rate is not steady in its increase corresponding with the age groups For example, it remains practically constant from the sixth group to the twelfth group (Table III) Although the microfilaria rate might be expected to rise proportionately with the period of exposure, such a rise is not apparent This would appear to be due to the phenomenon of disappearance of microfilariæ in infected individuals consequent to the onset of filarial disease That seems to be the reason why, in spite of the steady increase observed in the filarial disease rate and the filarial endemicity rate in the higher age groups, the microfilaria rate remains more or less constant

Intermediate host

To determine the transmitter of filarial infection in Trivandrum, a large number of mosquitoes collected from dwelling houses in different parts of the city were dissected and examined for filarial infection Over 5,600 specimens of different species were examined during the period October to December 1931, and the findings are furnished in Table IV —

Table IV

Results of mosquito dissections

Species	Number examined	Number positive
Culex fatigans Wied	5,477	1,128
Culex vishnui Th	28	0
Culex gelidus Th	3	0
Culex sitiens Wied	6	0
Culex bitæniorhynchus Giles	2	0
Lutzia fuscanus Wied	2	0
Armigeres obturbans Walk	25	0
Mansonia (Mansonioides) annuliferus Th	20	1
M (M) uniformis Th	2	0
Anopheles vagus Don	6	0
Anopheles subpictus Grassi	18	0
Anopheles barbirostris Wulp	1	0
Anopheles culicifacies Giles	1	0
Anopheles tessellatus Th	1	0

Only two species were found naturally infected, namely *Culex fatigans* and *Mansonia (Mansonioides) annulifera* The observations on *Culex fatigans* are based on a very large number of examinations, developmental phases of *W bancrofti*, mature larvæ or both were observed in 1,128 specimens out of 5,477 examined, working out to a gross natural infection rate of 20 3 per cent The observations on *M annulifera* are based on a comparatively small number of examinations Out of 20 specimens of this species examined, one was found infected The filaria larvæ found in the single infected specimen were in a very early stage of development, later stages of development were not observed in this mosquito Sufficient evidence is not at hand to show that *M annulifera* plays a part in the transmission of the infection in Trivandrum Judging from the numerical prevalence of *Culex fatigans* and the high rate of infection observed under natural conditions, it is evident that this species is the prime, if not the sole, agent concerned in the transmission of filarial infection in Trivandrum This is further demonstrated by the observation that the extent of breeding of *Culex fatigans* and the incidence of adult mosquitoes in dwellings are heaviest in areas with high microfilaria rates, and that areas with a low incidence of adult *Culex fatigans* have low microfilaria rates

Intensity of infection among *Culex fatigans*

The natural infection rate among *Culex fatigans* varies considerably in different areas in Trivandrum The results of the examination of *Culex fatigans* collected from dwelling houses in different sections of Trivandrum (Table V) show that the natural infection rate in *Culex fatigans* bears a close relation to the extent of infection among the human population —

Table V

Sections	Number of *Culex fatigans* examined	Number found infected	Infection rate per cent
Fort I	300*	81	27 0
„ II	90	27	30 0
„ III	98	43	43 8
Manacaud I	1,500*	329	21 9

TABLE V—*concld*

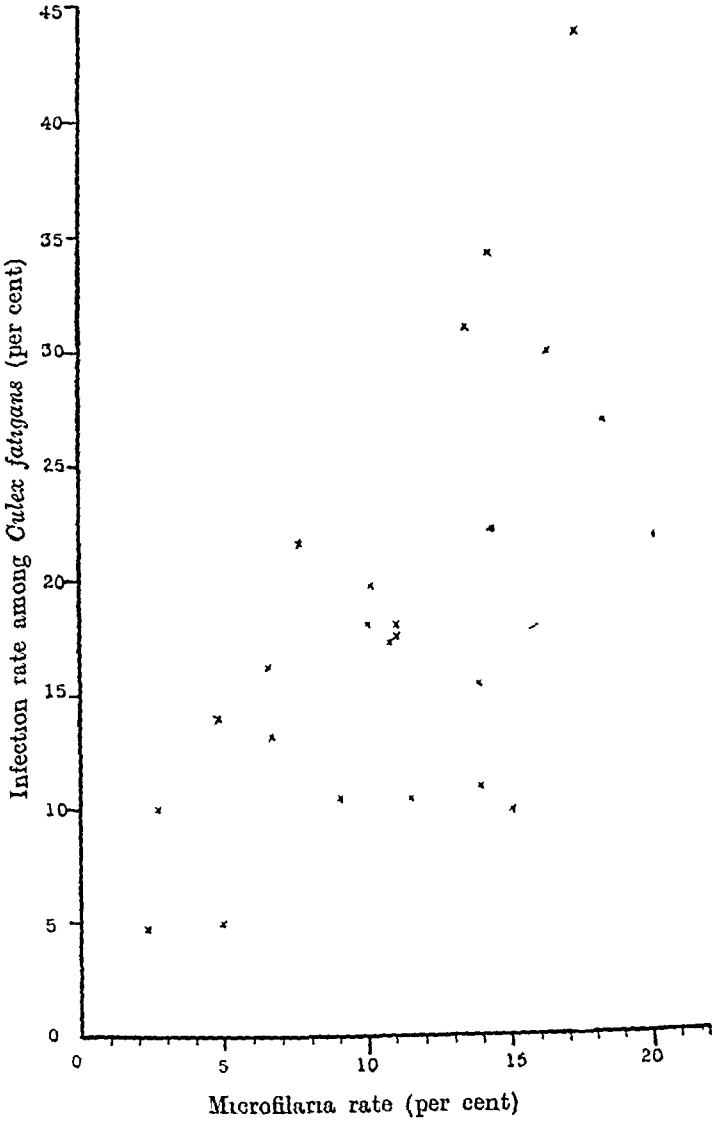
Sections	Number of *Culex fatigans* examined	Number found infected	Infection rate per cent
Manacaud II	142	14	9 9
, III	136	24	17 6
Chalai I	193	38	19 7
II	188	42	22 3
, III	162	25	15 4
, IV	200	22	10 5
, V	540*	185	34 3
Puthenchandai I	143	19	13 3
II	156	17	10 9
III	181	39	21 5
Pettah I	151	47	31 1
II	239	41	17 2
, III	101	5	5 0
„ IV	120	12	10 0
„ V	178	32	18 0
Vellayambalam I	113	15	10 5
, II	108	15	13 9
„ III	189	34	18 0
„ IV	105	17	16 2
, V	105	5	4 8
TOTALS	5,477	1,128	20 6

*This work of the examination of the mosquitoes was carried out in greater detail in three sections, Fort I, Manacaud I and Chalai V which are represented by a larger number of observations

A comparison of the mosquito infection rates (Table V) with the microfilaria rates of the respective sections (Table I) brings out this relation In areas with

high microfilaria rates, the infection rate among *Culex fatigans* is high and vice versa The positive correlation between the mosquito infection and human infection is shown graphically in Chart 4 and expressed by the formula, $r = +0.5948 \pm 0.0888$ These results are what one would naturally expect, namely, that the

CHART 4



infection rate among the transmitting intermediate host varies directly as the incidence of carriers among the human population

SUMMARY

1 Microfilariæ observed in man in Trivandrum are those of *W bancrofti*

2 The gross microfilaria rate of Trivandrum is 10 5 per cent The flat low-lying area in the centre of the city has microfilaria rates of 15 to 20 per cent The outskirts of the city have low infection rates

3 A combination of the microfilaria rate and the filarial morbidity rate is a better measure of filarial endemicity than either of the two data by themselves

4 The gross filarial morbidity rate is 33 1 per mille The central areas of Trivandrum have high filariasis rates (over 70 per mille) The incidence of filariasis in the outlying areas is comparatively low Elephantiasis of the limbs is the most common affection and constitutes 53 per cent of the filarial affections

5 There is a high degree of positive correlation between the microfilaria rates and the filarial morbidity rates Areas with a high microfilaria rate have a high incidence of filariasis, and vice versa

6 The chances of finding microfilariæ in filarial subjects vary inversely as the duration and severity of the affection Microfilariæ are comparatively more frequent in early stages of filarial disease, such as lymphangitis, than in later stages, such as elephantiasis of the leg

7 The earliest age for the presence of microfilariæ in peripheral blood as observed in Trivandrum is $2\frac{1}{4}$ years, for the occurrence of lymphangitis 8 years and for elephantiasis of the leg 11 years

8 There is a fair degree of positive correlation between the microfilaria rate and the average infestation (average number of microfilariæ per positive film)

9 Filariasis rate and filarial endemicity rate vary directly with age The microfilaria rate also shows a rise with age but, after the 25th year, it is nearly at the same level

10 *Culex fatigans* is the transmitter of the infection in Trivandrum The natural infection rate in *Culex fatigans* varies in the different sections of Trivandrum and bears a close relation to the extent of infection among the human population

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The survey of the incidence of filariasis in Trivandrum was inaugurated by Dr W P Jacocks, M D , Honorary Adviser, Public Health, Travancore, and was carried out under his guidance till the writer took charge of it in 1931 The writer is greatly indebted to Dr Jacocks for his kindness in letting the writer have the use of the prior records of the survey and for the facilities he gave in connection with this investigation The collection of blood films and of field data on incidence of filariasis was made by the medical staff of Dr Jacocks Particular mention

should be made of Dr M I Mathew, Sub-Assistant Surgeon, who did the major part of the field work A large part of the laboratory work was carried out by Miss K C Martha, Laboratory Technician The writer desires to acknowledge his indebtedness to these workers for their assistance and co-operation and to Mr V A Nair, Statistical Clerk, for working out the correlations referred to in the article

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CHOLESTEROSIS OF THE GALL-BLADDER IN INDIANS

A CLINICAL STUDY IN HUMAN SUBJECTS AND AN EXPERIMENTAL STUDY IN RABBITS

BY

A C GHOSE, MB, BS,

Captain Kunwar Indrajit Singh Research Scholar

(From the Department of Surgery, Lucknow University)

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INTRODUCTORY

IT IS evident from the literature that the incidence of the inflammatory and calculous diseases of the biliary system is very common in Occidentals, while Morse (1927) remarked that it is not a prevalent disease in Orientals Indians are, by no means, immune to the disease Owing to the lack of statistical reports no definite inference could be drawn as regards its incidence, but it seems to be not infrequent in Indians Females suffer from this ailment at least three times more than the males in the western countries, and it is difficult to assign any one cause for such sex disproportion There is no such sex disproportion in the incidence of gall-bladder diseases in Indians, here both sexes seem to be equally subject to cholecystitis and gall-stones (Ghose, 1931)

If the signs and symptoms of gall-bladder diseases be prominent in a sufferer, no difficulty arises in diagnosing the condition But the diagnosis of a case of diseased gall-bladder may become very difficult indeed, if the patient complain of nothing else except an intractable dyspepsia, which is also a symptom of various organic lesions of the visceral organs, or of some mechanical interference in the passage of the fæces In spite of the clinical features of such a case not clearly indicating any of the common diseases of the gall-bladder, in spite of the various available clinical and radiological methods of diagnosis having been exhausted without any successful results in the diagnosis of gall-bladder disease, and in spite of a gall-bladder appearing normal to the naked eye and feel at an exploratory laparotomy, a few cases turn out to be a curious pathological condition of the

gall-bladder which gives rise to vague visceral sensations and abdominal discomforts This disease of the gall-bladder has recently been named 'cholesterosis' of the gall-bladder, and is now coming into prominence as a clinical entity by itself in the surgical diseases of the biliary system It is now claimed that such a pathological condition is found in about one-fifth of all types of diseased gall-bladders removed at operation (Illingworth, 1930)

Historical

Moynihan (1909) first described yellowish spots, like sand or grit, on the mucous membrane of the gall-bladder, and called attention to the presence of cholesterol crystals in the bile in such a case MacCarty (1910) was the first to give this condition the name of 'strawberry' gall-bladder, from the resemblance which the tiny yellow specks, scattered over a reddish background, bore to a ripe strawberry He also recognized that the yellow deposits were lipoid in nature, because they stained red with Scharlach R Lichtwitz (1914) described the condition in detail and emphasized the fact that it was a not infrequent finding at operation He was probably the first to make the observation that the lipoid-like deposits were cholesterol

Boyd (1923) suggested the term 'lipoid' gall-bladder, which would include both the localized and generalized infiltration of lipoid in the gall-bladder, that may not be visible to the naked eye He confirmed the opinion of Lichtwitz and demonstrated by histological and chemical methods that the lipoid-like deposits were esters of cholesterol He supported the theory of the absorption of cholesterol by the gall-bladder, as suggested by Aschoff (1906), and believed that a disturbance in this absorptive function could explain the extensive cholesterol deposits in the organ

Mentzer (1925) recognized the 'patchy' and 'diffuse' types of lipoid infiltration of the gall-bladder, and named them 'cholesterosis' of the gall-bladder He also believed that the mucosa of the gall-bladder absorbs cholesterol Illingworth (1930) also accepted this view and added further knowledge, by clinical and experimental studies, to the ætiology of cholesterosis of the gall-bladder

The recent studies of Elman and Graham (1932) on the function of the gall-bladder give a probable explanation of cholesterosis of the gall-bladder They have brought forward evidence, by experiments on dogs, which show that the gall-bladder excretes (rather than absorbs) cholesterol, particularly in the presence of inflammation

Clinical significance

The reports by Moynihan (1909), MacCarty (1910), and Lichtwitz (1914) of cases necessitating cholecystectomy, in which the gall-bladder showed no gross

inflammatory lesions or stones in it, point to the fact that cholesterosis of the gall-bladder is capable of giving rise to symptoms which may be a constant source of trouble to the patient For a time its clinical significance was overlooked, and it may be that many such gall-bladders, which seem quite normal from their external appearances, were not tackled by the surgeon operating in the region of the gall-bladder

In 1923, Boyd quoted cases in the support of the view that cholesterosis does give rise to symptoms and Illingworth (1930) seems to have confirmed this In cholesterosis of the gall-bladder, an individual usually complains of mild pain and drawing sensations in the epigastrium There is no periodicity of the sensations of abdominal discomfort, there is no eructation of acid or of gas There may be nausea, and the loss of appetite may be progressive in nature, so that the patient loses weight Although the patient prefers to have a fluid diet or semi-solid food, yet there is not much relief of the abdominal discomfort and painful sensations in the epigastrium on this restricted diet The pain may be in the right hypochondrium, constant and distressing in character, but is by no means of the colicky nature, which would suggest the passage of a biliary calculi The absence of temperature and jaundice is an important clinical symptom

In this condition various diagnostic measures, such as, Leyon-Mentzer's duodenal intubation after magnesium-sulphate insufflation, examination by various hepatic efficiency tests, blood examination for leucocytic counts and X-rays, do not help much, i e, their negative results are not of much significance It will be shown later that cholesterol estimation of the blood may be of some utility in the diagnosis of this condition

There were 5 cases of cholesterosis of the gall-bladder and the above-mentioned clinical symptoms have been detailed by analysis of the signs and symptoms present in those cases There were 19 cases of diseased gall-bladder, which were operated upon, out of these, 14 cases showed gross inflammatory lesions with or without gall-stones Therefore we find that in about one-fourth of these cases the external appearance of the gall-bladder is normal, while the organ as a whole is diseased, so that it is no longer capable of carrying on its normal function

Morbid anatomy

The strawberry gall-bladder presents externally a more or less normal appearance, but the picture is very interesting when the gall-bladder is opened and an examination of the internal coat is made The normal appearance of the folds of the mucosa is altered, in marked cases dense yellow masses may be seen on a reddish background

The use of a dissecting microscope is of the greatest value in the study of this condition The slender and delicate gossamer folds of the mucosa are seen to be loaded with the dense and opaque lipoid material in 'strawberry' gall-bladder

In the mild cases the deposits are present here and there in the folds of the mucosa, while in the severe cases the distribution is widespread The opaque material is generally seen in the summit of the ridges and sometimes it can also be traced down into the recesses formed between the deep folds of the mucosa Out of 19 gall-bladders examined, 5 presented this picture in various degrees, and in one case there was a definite thickening at the fundus and a mass of tissue was embedded in the mucosa This was the so-called adenoma of the gall-bladder, probably in its early stage

The fundus is generally found to be more thickened than the neck of the gall-bladder The ridges at the fundus are usually low, thick and abnormal in appearance, while the ridges of the mucosa at the neck of the gall-bladder may look normal with slight opacity here and there

The presence of biliary calculi is not infrequent in cholesterosis Illingworth (1930) found gall-stones in nearly half of the cases in his series, and he mentioned that the stones either solitary or multiple were composed of pure or almost pure cholesterol Thus, a relationship between the deposit of cholesterol in the mucous membrane and the formation of cholesterol stones was clearly demonstrated

Histology

The minute anatomy of the diseased gall-bladder was studied by cutting sections, embedded in paraffin For the demonstration of lipoid, the sections were primarily treated with potassium bichromate solution and fixed in formol The staining was done with Scharlach R, Sudan III, and Lorrain-Smith's Nile-blue-sulphate methods

In this condition there were signs of inflammation in 4 out of 5 gall-bladders in which the wall of the gall-bladder was slightly thickened due to fibrosis The villi of the mucosa presented a frond-like appearance and were loaded with lipoid, which was mostly seen in the epithelium of the surface and the stroma of the villi or in both Such projections of the villi suggest two phenomena—either the mucous membrane by its supposed absorptive function has absorbed as much cholesterol as possible from the bile saturated with cholesterol (Illingworth, 1930), or the mucosa is simply loaded with lipoid which has accumulated there owing to defect in its probable excretory function which may have been brought about by the inflammation of the organ (Elman and Graham, 1932)

The substance of the mucous membrane, containing loose connective tissue and a large number of mononuclear cells due to mild inflammation, was found to be filled with lipoid, which gave it a granular appearance

The capillaries of the villi were not found to contain lipoid, but Boyd (1923) found the blood vessels of the muscle coat contained cholesterol, which he thought to be evidence, which, although not conclusive, was nevertheless suggestive of the possibility that the lipoid is absorbed into the blood vessels

The lipoid was also seen in the fibro-muscular coat, but not so abundantly as in the mucosa The young fibro-blastic cells were often found loaded with granules, but the lipoid could be seen extra-cellularly as well It may be granular in form or as narrow streaks, which suggests that the lipoid is confined within the tissue spaces or lymphatics

The sub-serous layer was not free from the lipoid deposits, they were found in large number in one case, in which the crystals of cholesterol could be demonstrated in the muscular and sub-serous layers of the gall-bladder

To summarize the observations on the distribution of the lipoid, it is most common in the mucosa, but may also be present in the deeper layers, it is generally found in granular form as cholesteryl-esters, but may be present in crystalline form as well

Pathogenesis

An explanation of the extensive deposits of cholesterol in the gall-bladder was given by Boyd (1923) These he thought were due to the defective absorption of cholesterol by the mucous membrane of the gall-bladder This theory of the absorptive function of the mucosa was first given by Aschoff (1906), and later on was supported by Mentzer (1925) and Illingworth (1930)

Most of the workers observed that there is always some inflammatory signs in the gall-bladder in this condition Illingworth (1930) further added that infection is essential for the production of cholesterosis of the gall-bladder, because with hyper-cholestræmia alone deposits of cholesterol in the gall-bladder were in minute traces only, while it was shown by him, experimentally, that intramural infection of the gall-bladder in rabbits on a diet high in fat could produce 'strawberry' gall-bladder Chiray and Pavel (1927), Lecene and Moulonguet (1926) and others regard the inflammation of the gall-bladder as the cause of cholesterosis Gosset *et al* (1921) and Mentzer (1926), however, have noticed the absence of infection in cholesterosis of the gall-bladder Blaisdell and Chandler (1927) have, experimentally, upheld the view that hyper-cholestræmia alone may cause such cholesterosis, because they have shown that in rabbits a prolonged course of feeding with cholesterol leads to deposits of this substance in the gall-bladder Dewey (1916) also found deposits of lipoid in the gall-bladder after long continued injections of cholesterol Stewart (1915, 1924), like many others, has regarded cholesterosis as a result of hyper-cholestræmia

It was for the purpose of determining the correlation of hyper-cholestræmia and infection in cholesterosis of the gall-bladder that these clinical and experimental studies were undertaken

(A) *Clinical studies in human subjects*

The cholesterol content of the blood was estimated by the method of Bloor (1916) For the estimation of cholesterol in the bile, 2 c c of bile was dried in 5 g

plaster of Paris and an alcohol-ether extract was made which was evaporated to dryness and the residue then dissolved in chloroform, the Leibermann-Burchard reaction was used for the colorimetric estimation The cholesterol content of the gall-bladder was estimated by the recent method of Osato and Heki (1930)

Table I shows that the cholesterol content of the blood and bile is increased to some extent in cholesterosis but the increase of the lipoid in the gall-bladder is enormous The crystals of cholesterol were also found in most of these cases

TABLE I

Cholesterol content of the blood, bile and gall-bladder in the human subjects

Case number	Blood cholesterol mg per cent	Bile cholesterol mg per cent	Gall-bladder cholesterol g per cent	Condition of the gall bladder
25	260	665	20 527	Strawberry gall-bladder, crystals of cholesterol present in the bile, also signs of inflammation
24	246	355	13 490	Do
9	220	474	16 230	Do
4	280	550	5 750	Do
18	210	670	8 620	Strawberry gall bladder, no signs of inflammation present
2	235	440	0 550	Sub acute inflammation on a fibroid gall bladder containing stones
16	332	465	0 750	Do
21	280	320	0 640	Fibroid gall bladder containing stones
	180	300	2 540	Maximum normal values

The signs of inflammation were present in all the cases save one The results of culture of the bile and the sub-mucous coat of the gall-bladder were positive in 3 out of 5 specimens of bile and in 1 out of 5 cultures of the gall-bladder, *Bacillus coli* was the common organism found

(B) *Experimental studies in rabbits*

The rabbits were divided into three groups Those of Group 1 received 200 mg of cholesterol daily—half the number by oral administration for 12 weeks and half by intra-peritoneal injections of 200 mg of cholesterol in 5 c c of olive oil for a period of 4 weeks To the rabbits of Group 2 cholesterol (200 mg daily) was given

orally and infection was produced by intravenous injections of *B coli* The animals of Group 3 received six weekly injections of *B coli* by the intravenous route

The results of the experiments are given in Tables II, III and IV The cholesterol content of the blood and gall-bladder in the rabbits of Group 1 (Table II), in which hyper-cholestræmia was produced, was found to be increased The values were higher than those obtained in the rabbits of Groups 2 and 3 The picture of the mucosa of the gall-bladder of the rabbits belonging to Group 1 showed, more or less, the same characteristic pathological changes (under dissecting microscope) as is seen in cholesterosis of the gall-bladder in human subject

TABLE II

Cholesterol content of the blood and gall-bladder in rabbits after the production of hyper-cholestræmia

Rabbit number	Blood cholesterol mg per cent	Gall bladder cholesterol mg per cent	Condition of the gall bladder
11	140	550	Gall bladder full of thick mucoid bile with masses of fats and debris, no signs of inflammation present
14	180	610	Do
15	140	600	Do
16	132	650	Do
17	142	540	Do
27	168	350	Do
28	160	400	Do
31	200	435	Slight inflammatory changes present

The average value of blood cholesterol in rabbit was found to be 78 mg per cent The average value of cholesterol in the gall-bladder of rabbit was found 300 mg per 100 g The maximum value was 357 and the minimum was 220

TABLE III

Cholesterol content of the blood and gall-bladder in rabbits after hypercholestræmia (by oral administration of cholesterol), and infection (by intravenous injections of B coli)

Rabbit number	Blood cholesterol mg per cent	Gall bladder cholesterol mg per cent	Condition of the gall bladder
36	102	320	Signs of inflammation present and debris in the bile
43	204	650	Bile was thick, mucoid, containing masses of fats
41	210	474	Bile was thick, mucoid, containing crystals of cholesterol Signs of inflammation present
37	108	325	Bile was thick, mucoid, crystals of cholesterol present, moderate inflammatory changes

TABLE IV

Cholesterol content of the blood and gall-bladder in rabbits after infection (by intravenous injections of B coli *isolated from a human gall-bladder suffering from chronic cholecystitis)*

Rabbit number	Blood cholesterol mg per cent	Gall bladder cholesterol mg per cent	Condition of the gall bladder
70	72	250	Gall bladder full of thick bile Early signs of inflammation present
77	70	320	Gall bladder shrunken and contained crystals of cholesterol Signs of inflammation present
79	68	310	Gall bladder contains thin greenish bile Signs of inflammation present
80	70	325	Gall-bladder contains thin bile with debris of greenish colour Signs of inflammation present

There was also a slight increase in the cholesterol content of the blood and gall-bladder in the rabbits of Group 2, while the quantity in Group 3 showed no such increase In fact, the gall-bladders of the rabbits of Group 2, closely resembled in their pathological appearance the diseased gall-bladders seen in human subjects, while the gall-bladders of the rabbits of Group 3 (Table IV) bore no evidence of cholesterosis

The results show that hyper-cholestræmia is an essential factor in the production of this condition The infection of the gall-bladder seems to hasten the process, as noticed in the rabbits of Group 2, probably by interfering with normal functions of the gall-bladder so resulting in the accumulation of lipoid in the wall of the organ

Discussion

It is difficult to explain the development of cholesterosis of the gall-bladder on the theory of absorption of cholesterol by the mucosa of the gall-bladder, as suggested by Aschoff (1906) and supported by Boyd (1923), Mentzer (1925), and Illingworth (1930) The lymphatics are supposed to be the pathway of absorption of the gall-bladder and the inflammation is supposed to act to produce lymphatic obstruction in the process of cholesterosis This theory of lymphatic obstruction is probably not true, firstly, because Winkenwerder (1930) has shown, experimentally, that the blood vessels and not the lymphatics are the most common pathway of absorption from the gall-bladder, and secondly, Elman and Graham (1932) mention that Copher and others were not successful in producing cholesterosis of the gall-bladder by obstructing the lymphatics

Elman and Graham (1932) and Andrews *et al* (1931) have found that, after ligation of the cystic duct, there is an increase in the amount of cholesterol in the bile and in the gall-bladder, which suggests that the gall-bladder excretes (rather than absorbs) cholesterol As there were signs of inflammation in most of the gall-bladders examined by them, Elman and Graham concluded that the presence of inflammation accelerates the process

Treatment

The treatment of cholesterosis would be a simple affair for a surgeon, if he did not tell the patient or his relatives that the patient was suffering from gall-stones He should rather be given to understand that he is suffering from disease of the gall-bladder When the disease is in the gall-bladder, it is not enough to remove the results of that condition, whether concretions or infected bile, but the disease itself must be treated Drainage of the gall-bladder will not cure the disease, nor will it relieve the symptoms permanently Cholecystectomy, therefore, should be the treatment of all surgical diseases of the gall-bladder

Cholecystectomy is performed as a primary procedure and in two stages In this condition where there is no operative risk, a primary operation is desirable That the surgeons of the King George's Hospital prefer to perform the primary operation, with or without draining the common bile-duct, in this condition, can be judged from the fact that out of 19 cholecystectomies 17 operations were of a primary procedure There were 5 cases of cholesterosis of the gall-bladder and the remaining cases were of cholecystitis and gall-stones The mortality of the operation was *nil*

The outlook on the problem of the treatment of the diseased gall-bladder has changed It was formerly the custom to remove the gall-bladder, only if the surgeon considered that a gross pathological change had occurred in the organ which would render its normal function impossible, and this decision may have been arrived at only by external examination of the viscus It is now accepted and well appreciated that even if there be no gross inflammatory changes, which is usually evident from the external appearance, there may be crystals of cholesterol or tiny calculi embedded in the mucous membrane of the gall-bladder, or there may be a local thickening of the gall-bladder the so-called 'adenoma', not infrequently giving rise to attacks of pain and the usual secondary dyspepsia Therefore nothing short of removal of the diseased gall-bladder would suffice in any of these conditions

Summary and conclusions

1 Cholesterosis of the gall-bladder is found in about one-fourth of the total number of gall-bladder diseases It gives rise to clinical symptoms which can only be got rid of by the removal of the diseased organ

2 The morbid anatomy and the histology of this condition is described The presence of lipoid in the various coats of the gall-bladder was demonstrated by lipoid stains and is an interesting feature of cholesterosis of the gall-bladder Inflammatory changes are also present in most of the cases

3 There is a definite rise in the cholesterol content of the blood, bile, and gall-bladder in this condition, and therefore, a blood cholesterol estimation, as a routine method prior to the operation, is of some diagnostic importance

4 Hyper-cholestræmia, produced by prolonged feeding of rabbits with pure cholesterol and by intra-peritoneal injections of cholesterol, is capable of giving rise to the condition of cholesterosis of the gall-bladder

5 Inflammation of the gall-bladder, when associated with a hypercholestræmic condition, seems to hasten the disease

6 Cholesterosis of the gall-bladder could not be produced by the infection of the gall-bladder alone, i e , without being associated with hyper-cholestræmia

In conclusion, I wish to express my indebtedness to those who have made this research possible—to Professor R N Bhatia for the guidance in the work, to Doctor K S Nigam for the facilities afforded me in studying the clinical side of the subject and to Captain J G Mukerjee for much valuable help in the pathological work The cost of the research was defrayed from Captain Kunwar Indrajit Singh Research Fund

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ON THE BACTERIAL INFECTION OF THE GALL-BLADDER

A CLINICAL STUDY IN HUMAN SUBJECTS AND AN EXPERIMENTAL STUDY IN RABBITS

BY

A C GHOSE, MB, BS,

Captain Kumar Indrajit Singh Research Scholar

(From the Department of Surgery, Lucknow University)

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INTRODUCTION

BACTERIAL infection has been found to be associated with the production of gall-stones and various types of inflammation of the gall-bladder Staphylococci, *pyogenes* and *aureus*, *B coli*, *B typhosus*, streptococci and *B welchii* are the most frequent organisms found in the gall-bladder infection In chronic cholecystitis, Hartman (1903), Drennan (1922), Johnson (1925), Hurst (1926) and Williams and McLachlan (1930) found *B coli* to be the most frequent organism responsible for the disease, while Rosenow (1914), Illingworth (1927) and Wilki (1928) laid stress on the rôle of streptococci in producing intramural inflammation of the gall-bladder

In view of the rôle played by the micro-organisms in the inflammatory and calculous diseases of the gall-bladder, a study on the bacteriology of human gall-bladder and an experimental study in rabbits was undertaken, which would throw light on the ætiology of cholecystitis and production of gall-stones

CLINICAL INVESTIGATIONS

The gall-bladders and stones after removal were taken to the laboratory, covered in sterile gauze in a sterile bottle and the samples of bile were taken in sterile test-tubes A piece of the entire gall-bladder was washed in saline and cultured For the culture of the submucosa, the outer layer and the mucous membrane was peeled off, and it was washed in sterile saline and cultured in (*i*) blood-agar, (*ii*) glucose broth, and (*iii*) litmus milk and was incubated aerobically and anaerobically About 0 5 c c of bile was emplanted on to culture tubes as mentioned above The isolation of the organisms was done by re-implantation on Condradi's plates and tested by sugar reactions Motility of the organisms was

noted and smears were examined after staining by Gram's method The stones were washed very well in running tap-water for about 2 to 3 hours and then kept in 5 per cent carbolic saline for about an hour and in absolute alcohol for a few minutes The excess of the alcohol was burnt off and the stone was placed in a blood-agar tube to see if any growth took place after aerobic and anaerobic incubation If there was no growth, the stone was broken and a piece was taken from its centre and was cultured as described

The bacteriological examination was made in 24 cases The results of the investigation are given in Table I The bile was infected in 64 per cent of cases and was sterile in 36 per cent The organisms isolated were *B coli* in 75 per cent, and streptococci in 12 5 per cent, and 20 per cent of the total infection was of mixed type—the organisms being *B coli*, streptococci, staphylococci and other intestinal bacteria

When the entire gall-bladder (all coats) was cultured the results were positive in 63 6 per cent in this series and the culture of the submucous layer was positive in 54 5 per cent *B coli* was present in majority of cases (about 83 per cent), while streptococci was found in 25 per cent and staphylococci in 33 3 per cent of the total infections Out of 7 stones cultured, 3 stones were infected and 4 were sterile, in 2 specimens there was pure culture of *B coli* and in one staphylococci

TABLE I

Culture of the fluid-content of the gall-bladder, the wall of the gall-bladder and gall-stones

Tissues	Number of cases	Infected	Sterile	Organisms
Bile	25	16	9	10 *B coli*, 2 streptococci, 4 *B coli* and staphylococci and other organisms
Gall bladder (all coats)	22	14	8	8 *B coli*, 2 streptococci, 1 *B coli* and streptococci and other organisms 3
Gall-bladder (submucosa)	22	12	10	9 *B coli*, 2 streptococci, 1 *B coli* and streptococci and other organisms 2
Stones	7	3	4	2 *B coli*, staphylococci 1

The results obtained are in accordance with that of Blalock (1924), Friesleben (1928), Hartmann (1903), and Williams and McLachlan (1929) It is contrary to the results obtained by Rosenow (1916), Illingworth (1927) and Judd *et al* (1927) Wilki (1928) found the gall-bladder infected in 6 and sterile in 44 in a series of 50 cases He, however, found 45 positive cultures in 50 cases when he cultured the cystic gland He found that the frequent organism was a streptococcus

The infection of the bile and of the gall-bladder was found in equal percentage which is about 64 per cent No evidence is found that one or the other coat of the gall-bladder is more prone to infection

The coliform bacilli are the most frequent organism found in the bile and in the wall of the gall-bladder All the coliform bacilli isolated were as a routine method inoculated into tubes of glucose, saccharose, lactose, dulcite, mannite and litmus milk in order to see the reaction and fermentation They usually showed the reactions of 'typical *B coli*', except in two cases where lactose fermentation failed to occur, an acid reaction took place with saccharose, and the identity of the organism was established as 'atypical *B coli*' This is in confirmation of the experience of Illingworth (1927) Besides *B coli*, streptococci and staphylococci, other intestinal organisms, such as *B enterıcus castellani*, lactic acid bacillus and *B carolinus castellani*, were also found in the cultures

FREQUENCY AND TYPE OF INFECTION IN VARIOUS STAGES OF CHOLECYSTITIS

Illingworth (1927) classified the affections of the gall-bladder into four groups, which was arrived at by the naked-eye appearances of the gall-bladder Such classification of the gall-bladder diseases was followed, and results are shown in Table II —

TABLE II

Location of infection in various types of cholecystitis

Group	Number of cases	Wall infected	Content infected
1 Acute cholecystitis	2	2	2
2 Chronic cholecystitis	10	7	9
With stones	8	6	7
Without stones	2	1	2
3 Early cholecystitis (chronic)	4	2	2
With stones	2	2	2
Without stones	2		
4 Cholesterosis	6	1	3

In acute cholecystitis the content and the submucous layer of the gall-bladder were infected by the coliform bacilli Stones were present in one case In chronic cholecystitis stones were present eight times and were absent in two *B coli* occurred in the bile in seven cases, in the wall, in pure culture in four and along with streptococci and other organisms in one, streptococci were present in two cases

Growth of *B coli* was found in the bile and in the wall of the gall-bladder—in two out of four cases of early cholecystitis Stones were present in two cases In cholesterosis of the gall-bladder the bile was infected by the coliform bacilli three times and the wall of the gall-bladder once

Experimental production of cholecystitis by injections of *Bacillus coli* isolated from human gall-bladder

Infection of the gall-bladder was produced in albino rabbits by repeated injections of *B coli* by the intravenous route *Bacillus coli*, which was isolated from the human gall-bladder, was cultured and made into an emulsion in saline About 150 millions in 1 c c was injected in the ear vein of rabbits, six such weekly injections were given The cholesterol content of the blood was examined at the interval of two weeks before, during and after the course of the injections The animals were killed and the gall-bladder was examined at various stages of the infection The maximum period that the rabbits were kept under observation was 20 weeks In the present work no attempt was made to obviate the possibility of a lymphatic spread of infection from the primary affection of the liver (mentioned by Graham, 1922), because Wilki (1929) has, experimentally, shown that after repeated venous injections of micro-organisms, the gall-bladder is infected through the cystic artery

Results—The results of the experiments are shown in Table III In four rabbits no inflammatory changes could be found after the first injection (rabbits 89 and 90) and second injections (rabbits 81 and 82) The rabbits (77, 79 and 80) which received three injections and other two (73 and 74) which received two injections suffered from mild type of cholecystitis—early signs of inflammation was found

Table III

Condition of the gall-bladder of rabbits during the course of injections of B coli *through the intravenous route*

Rabbit number	Culture of bile	Condition of the gall bladder
70	Positive *B coli*	Bile was dull greenish in colour, semi solid in consistency Signs of inflammation present
71	Positive *B coli*	Do
72		Do
73		Bile greenish, thin and watery No concretions, but a few epithelial cells present Early signs of inflammation present
74	Positive *B coli*	Do
79		Do
80		Do
77		Gall bladder shrunken and contained a few crystals of cholesterol Early signs of inflammation present
81		No abnormality in the bile or in the gall bladder
82		Do
89		Do
90		Do

in the gall-bladder In other rabbits (70, 71 and 72) there were signs of sub-acute inflammation in the gall-bladder and the bile was not normal in appearance, there were greenish masses of semi-solid consistency, and mucous epithelium was also present in the bile in large numbers

Twelve rabbits were kept under observation for about 14 to 16 weeks after the course of injections, and then they were killed for the examination of the condition of the gall-bladder The results are shown in Table IV In two rabbits (85 and 86) no abnormality was found in the gall-bladder, in another two (83 and 84) the bile was normal but the thickness of the wall of the gall-bladder and the projections of the villi of the mucosa were slightly abnormal The gall-bladder of the rabbits (69 and 78) was filled with greenish masses—the so-called 'mud',—and there were areas of cellular infiltration in the wall of the gall-bladder By the end of 12 weeks three rabbits (87, 88 and 68) were found to be ill, pyemic abscesses were found at the neck and at the back which contained a pure culture of *B coli* The gall-bladder contained muco-purulent fluid, and acute inflammatory changes were found in the gall-bladder In the remaining three rabbits (67, 75 and 76) the gall-bladder was distended with milky fluid There were a number of yellowish deposits which resembled the 'bilirubin calculi' found in human gall-bladder There were signs of inflammation in the gall-bladder

TABLE IV

Condition of the gall-bladder after a complete course of six weekly intravenous injections of B coli *in rabbits*

Rabbit number	Culture of bile	Condition of the gall bladder
67	Positive *B coli*	Gall bladder was distended with a milky fluid and yellowish deposits in large numbers present Signs of acute inflammation present
75	Positive *B coli*	Do
76	Positive *B coli*	Do
83		Bile was normal in colour and appearance Slight thickening of the gall bladder with signs of inflammation
84		Do
85		No abnormality was found
86		Do
69		Gall bladder filled with semi solid greenish masses—the so called 'mud' Mild signs of inflammation present
78		Do
87	Positive *B coli*	Gall bladder contained muco purulent fluid Acute signs of inflammation present
88	Positive *B coli*	Do
68	Positive *B coli*	Do

SUMMARY AND CONCLUSIONS

1 Bacterial investigations of bile wall of the gall-bladder and gall-stones were done both for aerobic and anaerobic organisms *Bacillus coli* was found in majority of cases There was no evidence that one or the other coat of the gall-bladder is more prone to infection

2 Cholecystitis has been experimentally produced by repeated injections of *B coli* isolated from human gall-bladder Acute cholecystitis, chronic cholecystitis and 'hydrops' of the gall-bladder have been found as the result of the injections Concretions in the gall-bladder are also found, a few of them are the so-called 'mud' composed of fats, and a few are bilirubin stones

In conclusion, I wish to express my indebtedness to those who have made this research possible—to Mr R N Bhatia under whose suggestions and guidance the work was started, to Doctor K S Nigam for the valuable instructions, guidance and facilities afforded me in carrying out the research and to Captain J G Mukerjee for much valuable help in the bacteriological and pathological work The cost of the research was defrayed from Captain Kunwar Indrajit Singh Research Fund

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THE INTERACTION OF FOOD AND SANITARY CONDITION IN THE CAUSATION AND PREVENTION OF THYROID DISEASE

BY

COLONEL R McCARRISON, CIE, KHP, IMS

(From the Nutritional Research Laboratories, I R F A, Pasteur Institute, Coonoor, South India)

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IN previous papers (McCarrison and Madhava, 1932, 1933) the existence of a goitre-noxa in association with insanitary condition was demonstrated anew (McCarrison, 1908, 1911, 1914, 1917, 1928) It was shown that albino rats, fed on a non-goitre-producing diet consisting of cabbage, whole wheat and *cholam* (*Andropagan sorghum*), developed goitre when living under conditions of gross insanitation but remained goitre-free when living under conditions of scrupulous cleanliness It was shown also that associated with the goitre produced by insanitary condition there was a significant increase in size of the adrenal glands and spleen and a significant diminution in size of the testes

The experiments, with which the present paper deals, were designed with the object of learning the nature of the effects of this noxa on the thyroid gland and the extent to which a properly constituted diet was capable of counteracting them

The experiments

The experiments were carried out during the spring, summer and early autumn of 1932, when the goitrogenic potency of cabbage was slight or *nil* They ran concurrently, and extended over a period of 181 days Seven groups of young albino rats were used, there being 36 animals in each group They were taken from the standardized stock maintained in this Laboratory Goitre is conspicuous by its rarity in this stock (McCarrison and Madhava, 1932*a*) In each group the body-weight of the animals ranged between 35 and 100 grammes, a range required to enable the effects of the goitre-noxa to be observed in animals at different stages of growth The aggregate body-weight and the sex-distribution in each group were approximately the same

Two diets were used a 'superior' and an 'inferior' The 'superior' diet consisted of freshly ground whole wheat flour, made into cakes of unleavened bread

(*chapattis*) and lightly smeared with fresh butter, sprouted Bengal *gram* (legume), fresh raw carrots and cabbage *ad libitum*, whole milk (5 c c per rat per day), fresh raw meat with bone once a week and distilled water The 'inferior' diet—so called because it was defective in certain obvious regards—was made up of 60 parts of fresh cabbage (the same cabbage was used in each diet), 20 parts of *cholam*, 20 parts of whole wheat flour (*chapattis*) and distilled water

The sanitary conditions under which the animals lived were also of two orders 'good' and 'bad' In the former each rat was confined in a separate, screened cage, the most scrupulous cleanliness being maintained In the latter the animals were confined together in two compartments of a single large cage (Plate XLI, fig 1) wherein rat excreta and decaying food had been allowed to accumulate from a previous experiment (McCarrison and Madhava, 1932), fermentation was favoured by the presence of yeast and the moist condition in which the decomposing material was kept The food of the animals became contaminated by this material

An emulsion of this excrement was made in distilled water 200 grammes to the litre Part of it—referred to as 'unsterile filtrate'—was filtered through several layers of muslin another part—referred to as 'sterile filtrate'—was filtered, under pressure, through microbe-proof porcelain candles (Cheavin's) A third part, also filtered through microbe-proof candles, had iodine added to it in the proportion of 10,000γ to the litre, this portion is referred to as 'sterile filtrate *plus* iodine' These filtrates were administered to three of the groups as their sole water-supply The filtrates were mixed with the food in the proportion of 10 c c per rat per day The group which received the sterile filtrate *plus* iodine was kept in an animal house far removed from the other groups

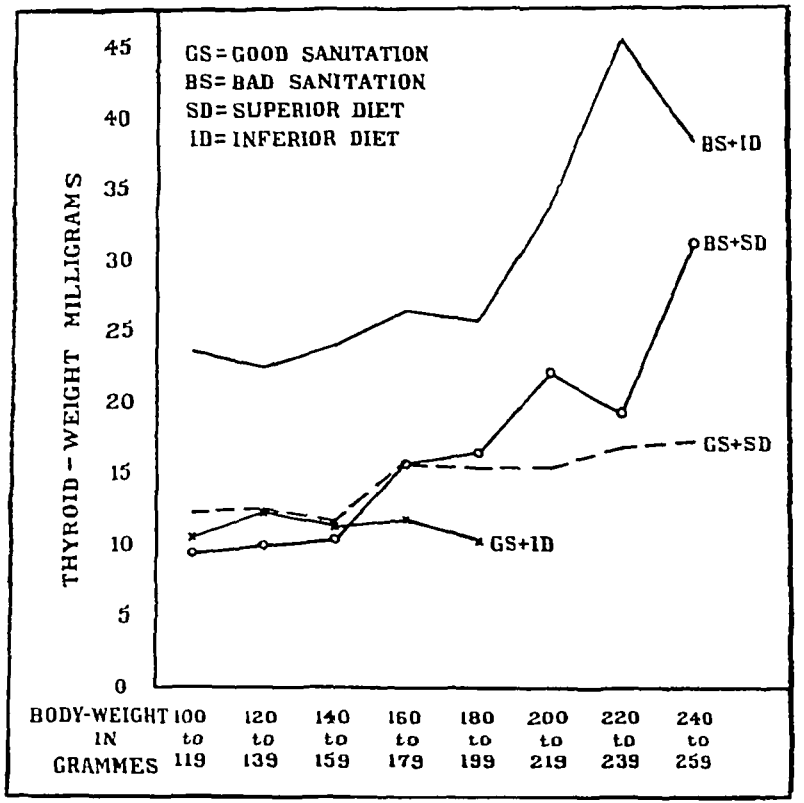
The conditions of life of the seven groups were thus as follows —

Group	I	good sanitation and superior diet
,,	II	good sanitation and inferior diet
,,	III	bad sanitation and superior diet
,,	IV	bad sanitation and inferior diet
,,	V	good sanitation, superior diet and unsterile filtrate
,,	VI	good sanitation, superior diet and sterile filtrate
,,	VII	good sanitation, superior diet and sterile filtrate *plus* iodine

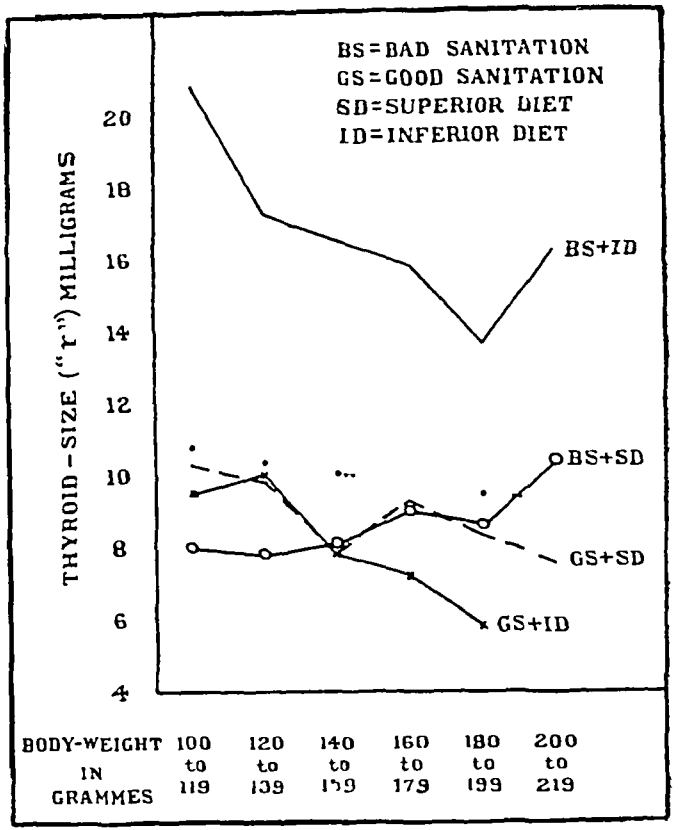
On the conclusion of the experiments the surviving rats were killed by drowning and the two thyroid lobes rapidly dissected out and weighed The isthmus was neglected, since its removal with the lateral lobes would have involved the expenditure of considerable time and subjected the main portion of the organ to the risk of drying Its exclusion does not materially affect the results

Results of the experiments.

The results are set out in Tables I and II, they are represented graphically in Text-figs 1 to 5



TEXT FIG 1 —Showing the weight curves of the thyroid gland in Groups 1, II, III and IV



TEXT FIG 2 —Showing the 'r' curves of the thyroid gland (weight per 100 g of body weight) in Groups I, II, III and IV The dotted line is the normal 'r' curve of stock rats in this laboratory

TABLE I

Showing the mean weights (T -W) and 'r'-values (weight per 100 g of body-weight), in milligrams, of the thyroid gland at different ranges of body-weight in albino rats living under conditions of good and bad sanitation while being fed on 'superior' and 'inferior' diets

Group—	I		II		III		IV	
	GOOD SANITATION				BAD SANITATION			
Body weight, g	Superior diet		Inferior diet		Superior diet		Inferior diet	
	T W	'r'	T W	'r'	T W	'r	T W	'r'
110—119	12 2	10 3	10 6	9 5	9 4	8 0	23 6	20 8
120—139	12 4	9 7	12 3	9 9	9 9	7 8	22 4	17 3
140—159	11 6	7 8	11 3	7 8	10 2	8 1	23 9	16 5
160—179	15 6	9 2	11 7	7 1	15 6	9 1	26 4	15 8
180—199	15 2	8 3	10 2	5 9	16 3	8 6	25 4	13 6
200—219	15 3	7 5			21 9	10 4	33 5	16 2
220—239	16 6	6 6			19 1	8 3	45 4	
240—259	17 0	6 2			31 0	12 2	38 2	
AVERAGES	14 5	8 2	11 2	8 1	16 9	8 6	29 8	16 7

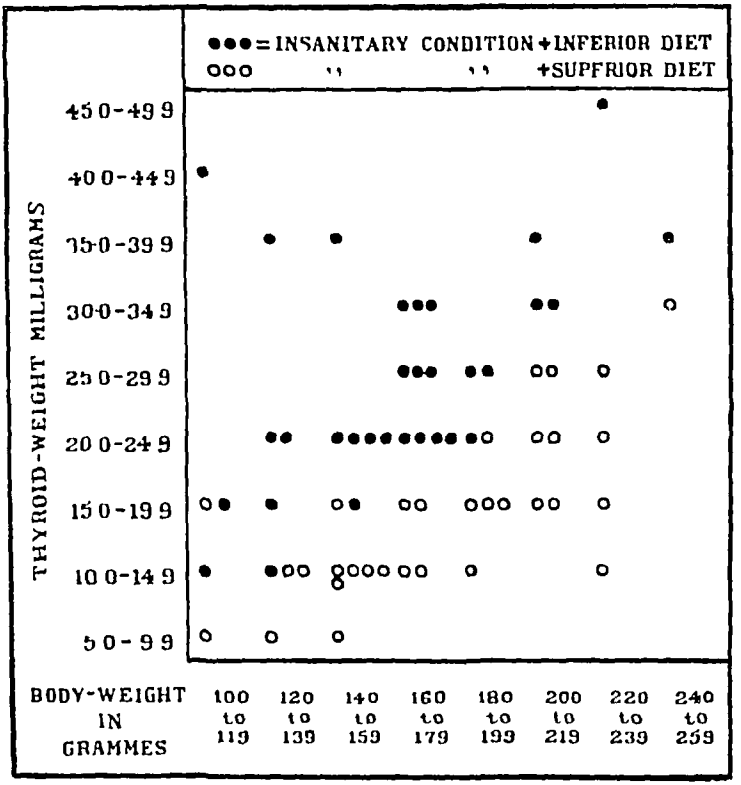
From these results the following facts emerge —

(1) The 'inferior' diet was not in itself goitre-producing (Text-figs 2 and 3), the mean value of 'r' being the same in Group II as in Group I This mean value is that (8 18±0 12) previously established as normal (McCarrison and Madhava, 1932*a*) for Coonoor rats

(2) The thyroid gland of rats fed on the 'inferior' diet was of significantly greater weight (T -W) and size ('r'), at every range of body-weight, when the animals were living under bad (Group IV) than when they were living under good (Group II) sanitary conditions (Text-figs 2 and 3) The existence of a goitre-producing noxa in association with insanitary conditions is thus demonstrated again

(3) Rats fed on the 'superior' diet while living under conditions of insanitation had thyroid glands of normal weight and size when their body-weights were less than 200 grammes The 'superior' diet

afforded them protection against the goitre-producing noxa associated with insanitary condition But in animals of a higher body-weight this protection, although considerable (Text-figs 1 and 2), was not complete It would seem, therefore, that despite the use of a well constituted diet, such as the 'superior' diet was, the goitre-noxa had a tendency to increase the weight and size of the thyroid gland in the heavier animals This tendency was not, however, so great



TEXT FIG 3 —Showing the frequency distributions of thyroid weights in Groups III and IV

as to cause 'true goitre', that is to say, the size ('r') of the glands did not exceed the normal mean by more than 2 5 times the standard deviation (McCarrison and Madhava, 1932*a*) The effect of the 'superior' diet in counteracting the goitre-noxa is illustrated in Text-fig 3

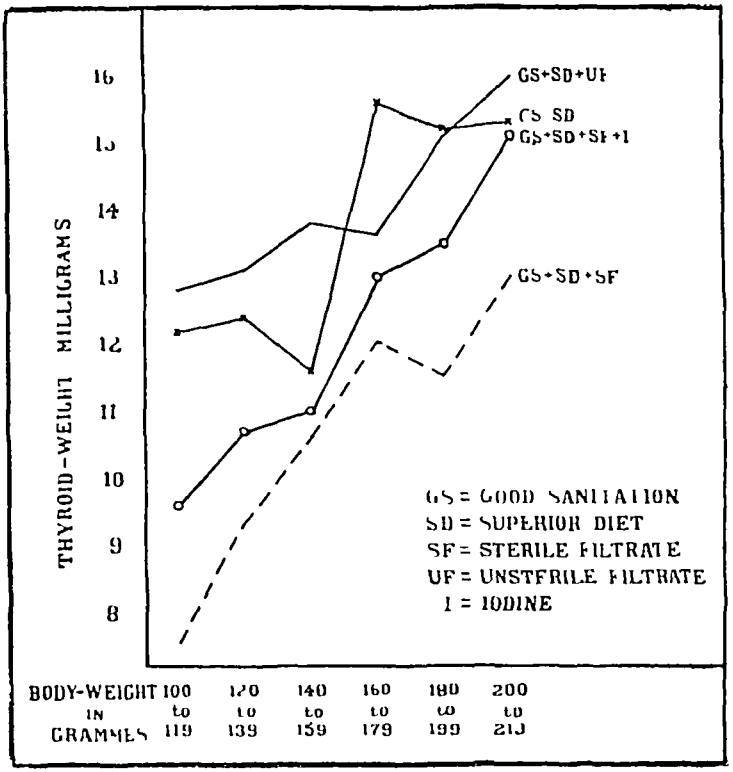
(4) The incidence of 'true goitre' in the several groups was as follows — I, nil, II, nil, III, nil, IV, 56 2 per cent

TABLE II

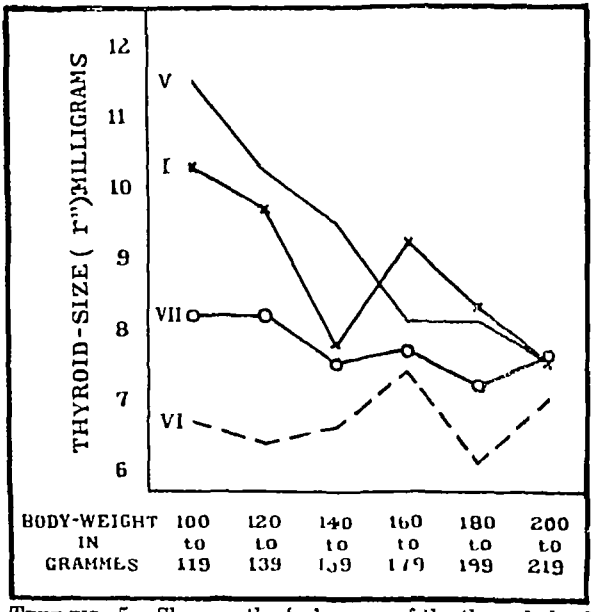
Showing the mean weights (T -W) and 'r'-values (weight per 100 g of body-weight), in milligrams, of the thyroid gland, at different ranges of body-weight, in albino rats living under good sanitary conditions, while being fed on the 'superior' diet to which the several filtrates were added

Group—	I		V		VI		VII	
Body-weight, g	No filtrate (control)		Unsterile filtrate		Sterile filtrate		Sterile filtrate *plus* iodine	
	T W	'r'	T W	'r'	T -W	'r'	T -W	'r'
110—119	12 2	10 3	12 8	11 5	7 5	6 7	9 6	8 2
120—139	12 4	9 7	13 1	10 3	9 3	6 4	10 7	8 2
140—159	11 6	7 8	13 8	9 5	10 6	6 6	11 0	7 5
160—179	15 6	9 2	13 6	8 1	12 0	7 4	13 0	7 7
180—199	15 2	8 3	15 1	8 1	11 5	6 1	13 5	7 2
200—219	15 3	7 5	16 0	7 5	13 0	7 0	15 1	7 6
AVERAGES	13 7	8 8	14 1	9 2	10 65	6 7	12 15	7 7

It is apparent from Table II that the 'unsterile filtrate' had no significant effect either on the weight (T -W) or the size ('r') of the thyroid gland in rats fed on the 'superior' diet while living under good sanitary conditions of life (Text-figs 4 and 5) And so far from the 'sterile filtrate' tending to cause thyroid enlargement it manifested a significant tendency, evident at almost every range of body-weight, to reduce the size of the organ This tendency was less marked, but still apparent, when iodine was added to the sterile filtrate than when it was not It will be noted that the diminution in size of the gland was most marked (Text-fig 5) at the lighter body-weights and, therefore, at the earlier ages, and that it gradually diminished as body-weight increased until at 200 to 219 grammes it was no longer apparent Indeed, at this—the highest—range of body-weight, the 'r'-values in all four groups were practically identical (7 0 to 7 6) It would seem, therefore, that the 'sterile filtrate' contained something which tended to reduce the size of the thyroid gland in the lighter and, therefore, younger animals



TEXT FIG 4 —Showing the weight curves of the thyroid gland in Groups I (Control), V, VI and VII



TEXT FIG 5 —Showing the ' r ' curves of the thyroid gland in Groups I (Control), V, VI and VII

A chemical analysis of the sterile and unsterile filtrates yielded the following results, expressed as grammes per litre.—

	Sterile filtrate	Unsterile filtrate
Total mineral matter	0 649	2 246
Insoluble	Faint trace	1 267
Soluble	0 649	0 979
Total nitrogen	Very faint trace	Trace (roughly double that in sterile filtrate)
Iron and alumin	Very faint trace	Trace
Lime (CaO)	0 112	0 074
Magnesia (MgO)	0 062	0 127
Manganese (Mn)	0 00005	0 00045
Potash (K_2O)	0 241	0 265
Nickel	Faint trace	*Nil*
Phosphates (P_2O_5)	0 106	0 467
Chlorides (Cl)	0 053	0 053
Fluorides (Fl)	*Nil*	*Nil*
Iodine (in γ per litre)	1 5	2 0
Sulphates	Faint trace	*Nil*

The faint trace of nickel in the sterile filtrate was derived from the porcelain candle through which the fluid was filtered, chemical analysis of the candle having revealed nickel in easily detectable amount. The possibility that it may have had something to do with the diminution in size of the thyroid gland in the younger animals is now under investigation.

Histological appearances of the thyroid glands.

Six sample glands were taken at random from each of the four groups (IV, V, VI and VII) in which the size of the gland led to the expectation of histological change of one kind or another.

The samples from Group IV (inferior diet and insanitation) were all goitrous. The goitres were of the same type—hyperplastic (Plate XLII, fig. 2). No retrograde changes, of the order presently to be described, were found in them.

PLATE XLI

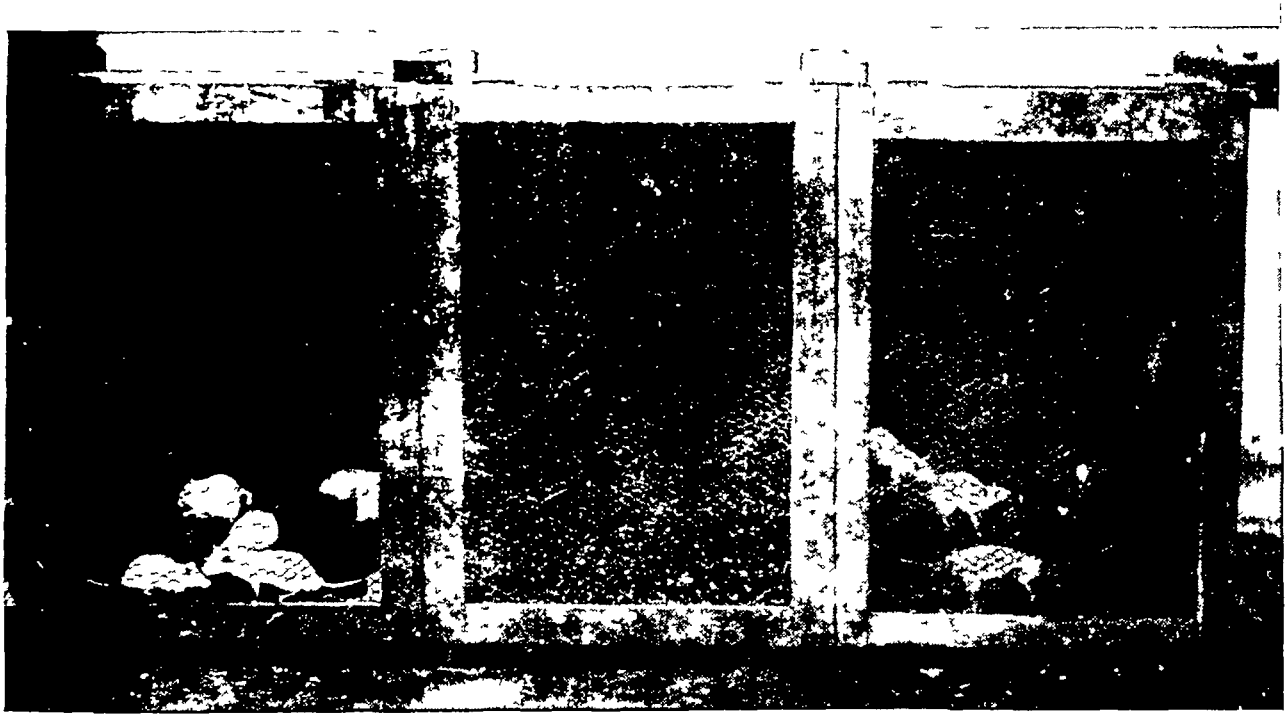


FIG 1 —Insanitary cage in which Groups III and IV lived, the middle compartment remained empty Cage turned on its side to show the compost of excreta and food remnants

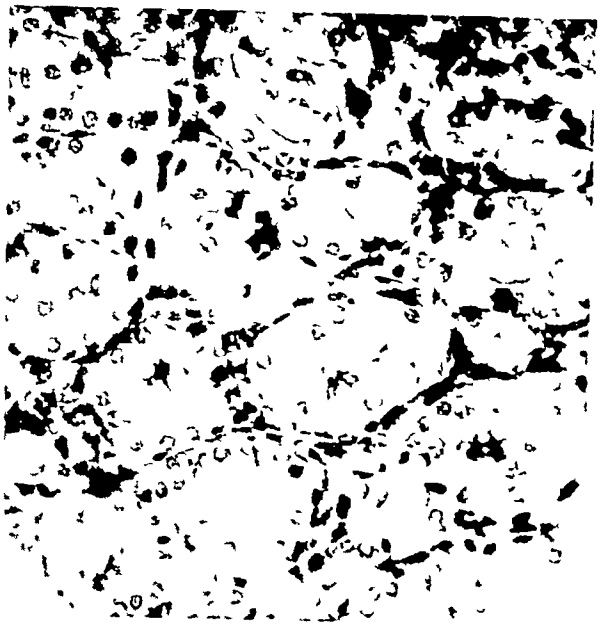


FIG 2 —Hyperplastic goitre typical of the enlarged thyroid glands of Group IV

FIG 3 —Non goitrous thyroid gland sample (No 3003) from Group VI Note both phases of normal activity active secretion and colloid storage

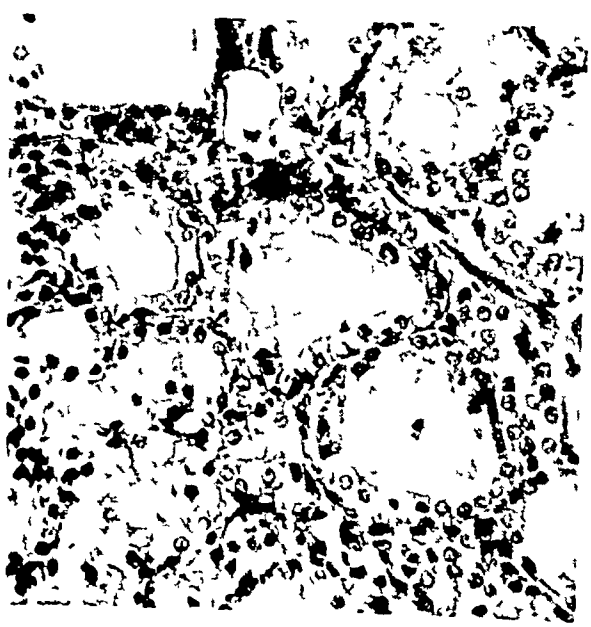


FIG 4 —Non goitrous thyroid gland sample (No 5103) from Group V Note commencing desquamation of epithelial cells, breaking down of follicle walls, some cellular disintegration

FIG 5 —Non goitrous thyroid gland sample (No 3098) from Group VII Note more advanced stage of the retrograde process which iodine administration did not prevent

FIG 6 —Non goitrous thyroid gland sample (No 5112) from Group V Note desquamation of epithelial cells, merging of colloid with cellular protoplasm aggregation of nuclei at certain areas

FIG 7 —Non goitrous thyroid gland sample (No 5103) from Group V Note epithelial cells mingled with colloid, and complete loss of follicular structure in lower part of the section

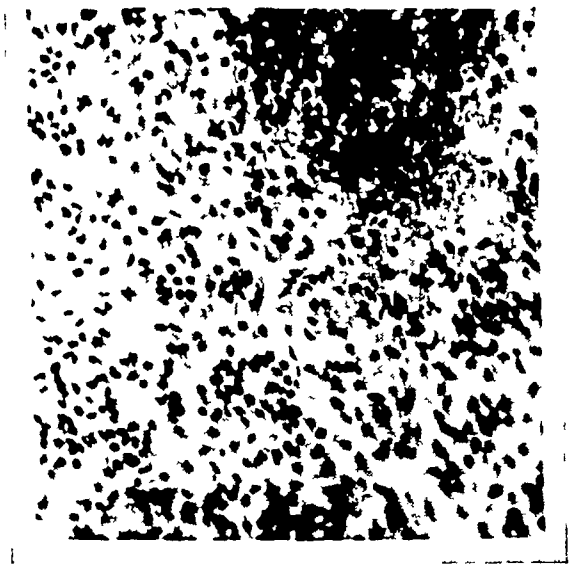


FIG 8 —Non goitrous thyroid gland sample (No 5069) from Group VI Note heaped up area of small, rounded, deeply staining nuclei and appearances resembling 'foetal adenoma' in the human gland

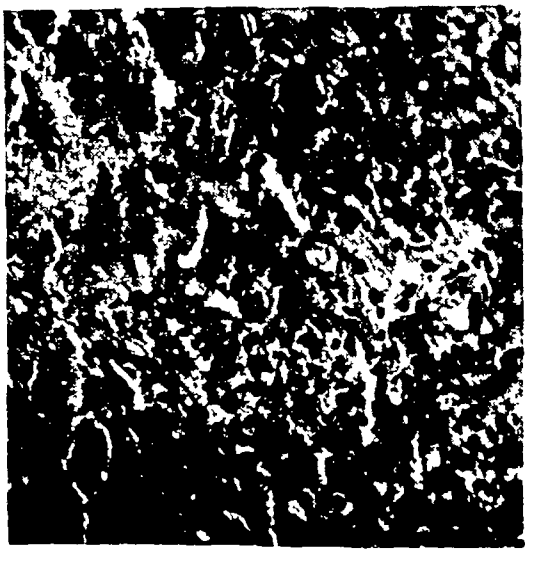


FIG 9 —Non goitrous thyroid , 5054) from Group VI Note a e seen in adenoma of the human

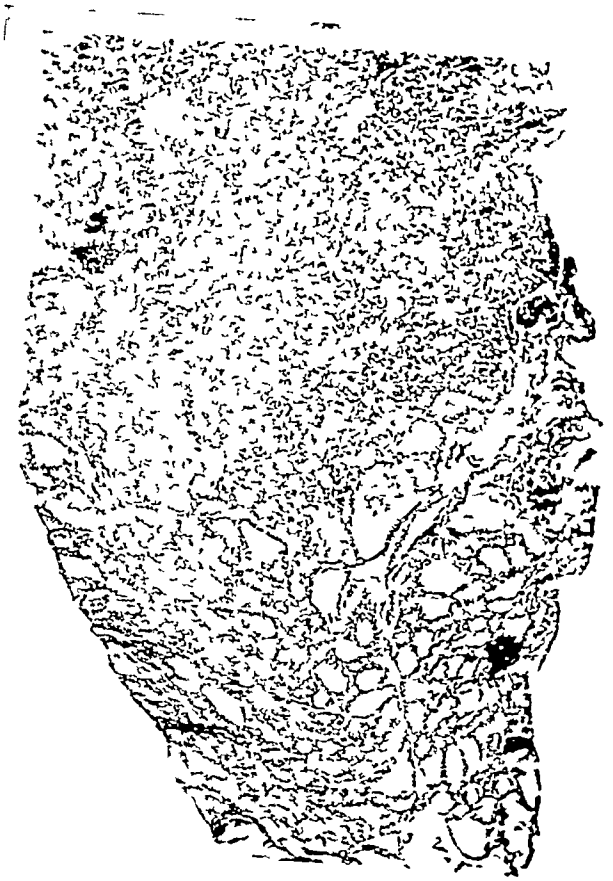


FIG 10.—Non goitrous thyroid gland sample (No 5102) from Group V. Note 'adenomatous' area in the upper part of the field, more or less normal area in the lower, and appearances suggestive of commencing encapsulation of the former area

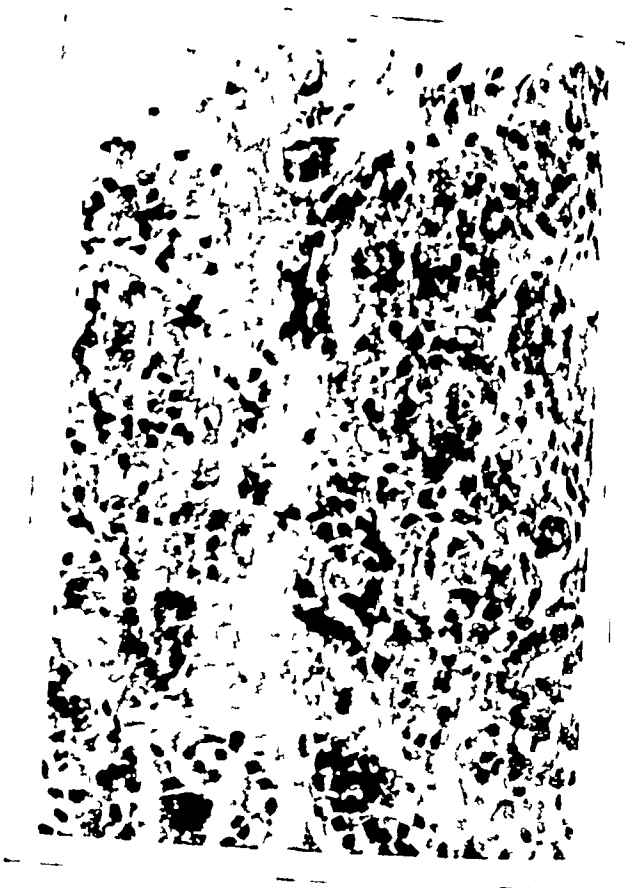


FIG 11.—High power view of part of the 'adenomatous' area seen in Fig 10

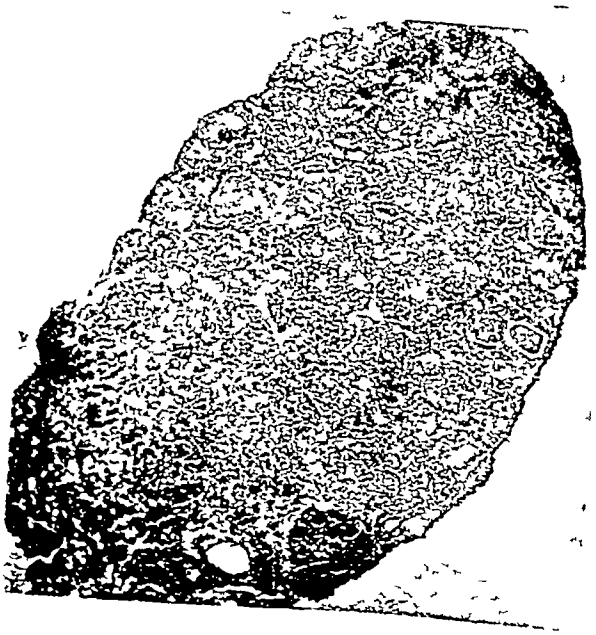


FIG 12.—Non goitrous thyroid gland sample (No 5097) from Group VII (sterile filtrate *plus* iodine). To show general appearances of the retrograde process and gland almost wholly involved therein

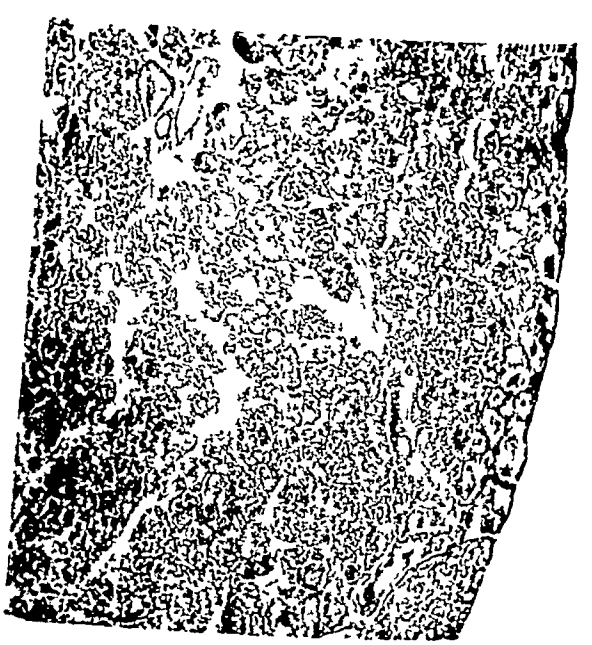


FIG 13. Non goitrous thyroid gland sample (No 5099) from Group VII (sterile filtrate *plus* iodine). To show appearances similar to those of the parenchymatous type of adenoma in the human thyroid: many small, closely packed follicles, for the most part empty of colloid

Those from Groups V, VI and VII (superior diet, good sanitation and filtrates) were of subnormal or approximately normal size They presented similar histological appearances in all three groups These appearances were of three orders normal, retrograde and a combination of both

Normal appearances —These need little description, they are illustrated in Plate XLII, fig 3 The gland exhibited both phases of its activity—colloid storage and active secretion—proceeding side by side with one another The phase of active secretion *usually predominated* This phase is sometimes spoken of as 'hyperplasia' in the literature It is admittedly difficult to distinguish it from hyperplasia by observation of a few fields of the microscope But where no enlargement of the gland has occurred the term 'active secretion' is preferable Some of these normal glands showed an unusual degree of distension of peripheral follicles with colloid material Of the glands that could be classed as 'normal' there were 3 out of 6 in Group VI, one out of 6 in Group V, and none out of 6 in Group VII

Retrograde changes —The gradual merging of the normal into the retrograde is illustrated in the accompanying photomicrographs (Plates XLII, figs 3 to 5, XLIII, 6 to 9, and XLIV, 10 to 13) Figs 4, 6 and 7 show what appears to be the first stage in the retrograde process an invasion* of colloid-containing follicles by epithelial cells, the breaking down of the follicular walls, the degeneration of certain cells, and the mingling of the unconfined colloid with the protoplasm of the follicular and intra-follicular epithelium Fig 10 shows these changes at the advancing edge of the process where it meets an area of normal glandular tissue In Figs 5 and 11 a further stage is seen here all trace of follicular structure and of colloid is lost, the epithelial cells are no longer distinguishable as individual units and their nuclei lie in a homogeneous or faintly granular and loosely disposed matrix, large portions of which are devoid, or almost devoid, of enmeshed nuclei, in such places cellular disintegration has occurred Figs 8 and 9 are examples of areas in which this matrix is more compressed enclosed within it are many, small, rounded, densely-staining nuclei more or less uniformly distributed or arranged in irregular clumps or heaped-up masses At this stage the appearances are similar to those seen in the so-called fœtal adenoma of human thyroids Figs 10 and 12 show, in low magnification, the general appearances of glands affected by this process In the former an appearance of commencing encapsulation of the adenomatous-like area is seen where it impinges upon a fibrous trabecula of the gland, in the latter the greater part of the gland is involved in the retrograde change, there being but one normal colloid-containing follicle to be seen in the section It can hardly be doubted

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* While the term 'invasion' appears adequately to describe this feature, 'desquamation' would also serve Such 'desquamation' is characteristic of the action of certain chemical and *microbic* poisons on the thyroid gland —R McC

that had the animals been allowed to survive for a longer period, with intermissions in the administration of the filtrates, the growth of fibrous tissue would have given rise to adenomatous nodules in glands affected by these changes

Fig 13 illustrates an appearance of a somewhat different order one closely resembling that seen in the parenchymatous type of adenoma in human thyroids, wherein are many small, closely-packed follicles mainly devoid of colloid

The incidence of these changes in the three groups (V VI and VII) receiving the filtrates did not differ significantly, nor did *their distribution* in the glands themselves In 3 of the 18 glands they were relatively slight and limited, as a rule, to the more central parts of the organ, in 11 others they were more extensive, occasionally they were confined to one lobe of the gland, or one lobe was more affected than the other Extensive retrograde changes were present in 4 out of 6 in Group V, in 3 out of 6 in Group VI and in 4 out of 6 in Group VII It will be noted that they were as frequent, and their distribution within the glands as extensive, in Group V (unsterile filtrate) as in Group VI (sterile filtrate) The presence of the trace of nickel in the 'sterile filtrate' was not, therefore, related to their occurrence, though whether the nickel caused the thyroid of the younger animals to be smaller than normal is another matter It will be noted also that the administration of iodine, simultaneously with the sterile filtrate (Group VII), did not prevent the occurrence of these changes

The retrograde changes here described have, at their later stages, a close resemblance to the so-called fœtal adenoma or, less often, to the parenchymatous type of adenoma in the human thyroid gland But whether or not they are to be regarded as adenomatous in nature they are obviously degenerative and such as must impair the functional efficiency of the gland There can be no reasonable doubt that they were due to a water-soluble noxa contained in the filtrates The *sensitivity* of the thyroid gland to this noxa, even in the presence of a well-constituted diet and/or of iodine, is evidenced by the occurrence of the changes in 12 out of 18, or 66 6 per cent, of the samples examined

As previously stated, changes of this character were not found in the hyperplastic goitres occurring in Group IV (inferior diet and insanitation) The number of samples examined (six) was, however, too small completely to exclude the possibility of their occurrence But it is evident that the administration of the water-soluble noxa was much more likely to give rise to them than the exposure of the rats to the insanitary conditions themselves It may be that the difference between Group IV and the groups receiving the filtrates was a matter of dosage of the noxa a smaller dose (as in Group IV) causing hyperplastic goitre, a larger dose (Groups V, VI and VII), causing the retrograde changes here described The latter groups consumed, daily, the equivalent of 2 grammes of the compost contained

in the insanitary cages, a dose which must have considerably exceeded that ingested by Group IV

Discussion

These results indicate (*a*) that, associated with the conditions of insanitation prevailing in these experiments, there was a *hyperplasia-producing* factor capable of giving rise to considerable goitres in rats fed on the inferior diet, (*b*) that the 'superior diet' contained a factor or factors antagonistic to this goitrogenic agent—*an anti-hyperplastic* factor—while the 'inferior diet' did not, or contained it in lesser amount, (*c*) that *a water-soluble noxa* was present in the compost of excreta and vegetable matter, which, when administered to well-fed and hygienically housed rats, caused retrograde changes of an apparently adenomatous kind, and (*d*) that the action of this noxa was not prevented by the administration of iodine in amounts equivalent to 100γ per rat per day

It has previously been demonstrated in this Laboratory (McCarrison, 1920, 1922, 1929, 1930) that hyperplastic goitres arising from such causes as insanitation, excess of fats in the food and an exclusive diet of cabbage, are preventable by the administration of iodine There can be little doubt, therefore, that the hyperplastic goitres which occurred in the animals fed on the 'inferior diet' while living under conditions of insanitation (Group IV) could have been prevented by iodine administration, just as they were prevented by the iodine-rich 'superior diet' (Group III) * But neither the superior diet alone nor in combination with iodine administration was capable of preventing the retrograde changes induced in the thyroid gland by the solutions of the noxa One is faced, therefore, with two possibilities either that the goitre-noxa associated with insanitary condition is a single entity, the action of which varies with its dosage and with the composition of the diet, or, that it is of dual nature, comprised of two elements—a 'hyperplasia-producing' one and an 'adenoma-producing' one

The evidence here presented affords no definite indication as to the nature of this noxa It is—or one element of it is—water-soluble, and therefore, water-borne In this connexion attention may be drawn to experimental findings of many years ago (McCarrison, 1911) Well-fed and hygienically housed goats were given, for a period of 107 days, a previously pure and non-goitre producing water which had been caused to percolate through a mixture of soil and fæces (the latter obtained from human sufferers from goitre) This was their sole drinking-water-supply It was found, on the conclusion of the experiment, that the thyroid glands of some of the animals were smaller than normal, a result similar to that obtained in certain groups of rats in the present experiments In another series of well-fed goats, cultures of fæcal bacteria from goitrous persons were administered daily

* Coonoor is situated in an iodine rich zone (McCarrison *et al*, 1927)

per os in massive dosage for a similar length of time (108 days) In these the numbers having unusually small thyroids were higher still, while histological examination of the glands revealed degenerative changes similar in kind to those found in Groups V, VI and VII of the present experiments In this connexion, also, the experimental production of goitre in adult animals and of congenital goitre and cretinism in their offspring by similar means (McCarrison, 1914, 1917) may be recalled

An investigation into the nature of the water-soluble poison is now in progress It is hoped to report upon it at a later date But, in view of the recent work of Marine (1932), and without attempting, at the present time, to draw any conclusion, it may here be said that the 'filtrates' yielded on acid distillation faint traces of cyanides, while the residue left after distillation was found to contain nitriles and amines of high molecular weight Alpha-hydroxy-carboxylic acids were present in the acid distillate, but no phenols nor other aromatic alcohols Indican, indole and skatole were not present Cadaverine was demonstrated in the 'unsterile' but not in the 'sterile' filtrate, putrescine was not found in either Tyramine, in relatively high concentration, and histamine were present Enzyme hydrolosis (emulsin), both of the compost and of the cabbage, revealed the presence of thio-cyanates in considerable quantity This observation, taken in conjunction with the traces of cyanide found in the acid-distilled fraction, would seem to suggest that the 'filtrates' contained thio-cyanate-precursors that are split up on acid hydrolosis into free cyanogen but which, on enzyme hydrolosis, yield thio-cyanate without further decomposition

Summary.

(1) Under the conditions of insanitation described in this paper a goitrogenic agent was evolved which caused hyperplastic goitres in the great majority of albino rats fed on a diet of poor anti-goitrogenic quality

(2) The well-constituted diet, described as 'superior', had definite anti-hyperplastic or anti-goitrogenic, qualities, this diet afforded albino rats, subjected to the above conditions of insanitation, a high degree of protection against hyperplastic goitre

(3) In the compost of excreta and decaying food accumulating under these conditions of insanitation there existed a water-soluble and water-borne 'noxa' which, when administered *per os* in daily doses of 10 c c of the watery solution, caused retrograde changes, of an apparently adenomatous nature, in the thyroid gland of albino rats

(4) These retrograde changes were not prevented by the well-constituted ('superior') diet, nor by the administration of iodine simultaneously with the watery solution of the noxa

(5) The goitre-noxa associated with insanitary condition may be of dual nature comprised of a hyperplasia-producing factor and an adenoma-producing factor The former is counteracted by a well-constituted diet and by iodine, the latter (in the doses in which it was administered) is not

Or, the noxa may be a single substance whose action on the thyroid gland differs with its dosage its ingestion in larger amounts over long periods causing retrograde changes in the organ even in the presence of a well-constituted diet and of iodine, its ingestion in smaller amounts causing hyperplastic goitre but only in the presence of an ill-constituted diet

(6) The interaction of food and sanitary condition on the causation or prevention of thyroid disease is again made evident by the results here recorded, and the limitations of a perfectly constituted diet and of iodine in counteracting this goitre-noxa are indicated

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Idem (1911)	*An Trop Med Parasit*, **5,** p 453
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Ind Jour Med Res, XX, 4, *April*, 1933

HYDROGEN ION CONCENTRATION IN THE ORGANS AND BODY-FLUIDS OF SCORBUTIC GUINEA-PIGS

BY

R McCARRISON AND G SANKARAN

(*From the Nutritional Research Laboratories, I R F A, Coonoor*)

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Material

Two groups of 25 guinea-pigs were used for this work The age, body-weight and sex-distribution of the animals was approximately the same in both groups One group ('Controls') was fed on a diet rich in vitamin C and made up of bran, green grass, fresh raw cabbage, fresh carrots, sprouted Bengal *gram* (legume) and water The other group ('Scorbutic') was fed on a diet of autoclaved milk and crushed oats in which vitamin C was lacking The experiment was continued until all the animals in the second group had developed clinical signs of scurvy Seven in this group died, these were discarded The remaining 18 were killed on the first appearance of scorbutic symptoms All the animals in the control group were likewise killed The blood needed for pH determinations was taken directly from the heart, the urine directly from the bladder All observations were made with the glass-electrode, within three hours of the death of the animals

Results.

The results are set out in Table I wherein the range of pH values and the mean pH are given, for each organ and fluid examined, together with the number of observations made

TABLE I

Showing the range of pH values and the mean value for the organs and body-fluids of healthy and scorbutic guinea-pigs

Organs	Healthy (Controls)			Scorbutic		
	Number of observation	Range of pH	Mean pH	Number of observation	Range of pH	Mean pH
Blood	23	7 36 to 7 55	7 51	17	7 17 to 7 47	7 32
Bile	17	6 71 „ 7 55	7 22	6	6 41 „ 7 16	6 99
Urine	12	6 72 „ 7 74	7 20	3	5·16 „ 6 74	5 68
Thyroid	24	6 88 „ 7 17	7 04	18	6 68 „ 7 02	6 90
Adrenals	24	6 28 „ 6 64	6 51	18	6 28 „ 6 57	6 47
Spleen	24	6 18 „ 6 59	6 45	18	6 30 „ 6 74	6 56
Auricle	24	6 05 „ 6 48	6 26	6	6 20 „ 6 74	6 60
Ventricle	24	5 70 „ 6 28	6 01	6	6 07 „ 6 64	6 49
Kidney	24	6 12 „ 6 68	6 40	17	6 12 „ 6 62	6 39
Liver	24	6 08 „ 6 71	6 37	18	6 22 „ 6 74	6 53
Testes	8	6 33 „ 6 79	6 54	7	6 30 „ 6 57	6 43
Cerebellum	23	6 28 „ 6 74	6 52	2	6 65 „ 6 65	6 65
Cerebrum	23	6 33 „ 6 91	6 54	2	6 65 „ 6 70	6 65

The frequency distributions of pH values are given for the blood, thyroid gland and bile in Table II , for the adrenal glands, spleen, kidney, liver and testes in Table III , and for the heart (auricle and ventricle) in Table IV

TABLE II

Showing the frequency distribution of pH values of the blood and thyroid gland and bile in healthy and in scorbutic guinea-pigs

Range of pH	Blood		Thyroid		Range of pH	Bile	
	Healthy controls	Scurvy	Healthy controls	Scurvy		Healthy controls	Scurvy
6 60 to 6 69				1	6 40 to 6 49		1
6 70 „ 6 79					6 50 „ 6 59		
6 80 „ 6 89			2	7	6 60 „ 6 69		
6 90 „ 6 99			2	8	6 70 „ 6 79	1	1
7 00 „ 7 09			13	2	6 80 „ 6 89	1	2
7 10 „ 7 19		1	7		6 90 „ 6 99		
7 20 „ 7 29		5			7 00 „ 7 09	2	2
7 30 „ 7 39	2	8			7 10 „ 7 19	3	
7 40 „ 7 49	4	3			7 20 „ 7 29	4	
7 50 „ 7 59	17				7 30 „ 7 39	3	
7 60 „ 7 69					7 40 „ 7 49	1	
					7 50 „ 7 59	2	
TOTALS	23	17	24	18	TOTALS	17	6

TABLE III

Showing the frequency distribution of pH values of the adrenals, spleen, kidney and liver in healthy and in scorbutic guinea-pigs

Range of pH	ADRENALS		SPLEEN		KIDNEY		LIVER		TESTES	
	Healthy controls	Scurvy	Healthy controls	Scurvy	Healthy controls	Scurvy	Healthy controls	Scurvy	Healthy controls	Scurvy
6·00 to 6·09							1			
6·10 „ 6·19			1		3	1	6			
6·20 „ 6·29	1	1	1		3	4	3	1		
6·30 „ 6·39	1	4	1	2	7	4	2	2	1	3
6·40 „ 6·49	6	4	13	2	3	3	5	5	3	2
6·50 „ 6·59	13	9	8	8	5	4	4	3	2	2
6·60 „ 6·69	3			3	3	2	2	2	1	
6·70 „ 6·79				3			1	5	1	
TOTALS	24	18	24	18	24	18	24	18	8	7

TABLE IV

Showing the frequency distribution of pH values of the auricle and ventricle of the heart in healthy and in scorbutic guinea-pigs

Range of pH	AURICLE		VENTRICLE	
	Healthy controls	Scurvy	Healthy controls	Scurvy
5·70 to 5·79			3	
5·80 „ 5·89			1	
5·90 „ 5·99			5	
6·00 „ 6·09	1		8	1
6·10 „ 6·19	7		4	
6·20 „ 6·29	5	1	3	
6·30 „ 6·39	7			1
6·40 „ 6·49	4			
6·50 „ 6·59		1		1
6·60 „ 6·69		1		3
6·70 „ 6·79		3		
TOTALS	24	6	24	6

From these tables it is seen that in the scorbutic guinea-pigs (1) the urine tended to be markedly acid, (2) the blood, bile, testes and thyroid gland exhibited a tendency to diminution in alkalinity, (3) the spleen, heart (auricle and ventricle) and the liver tended to be less acid than normal, while (4) the pH of the adrenal glands, and kidneys was not significantly altered

These results are in striking contrast to those observed in pigeons fed on diets deficient in vitamin B (McCarrison *et al*, 1933)

REFERENCE

McCarrison, R *et al* (1933) *Ind Jour Med Res*, **20**, No 3, pp 739–756

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A RAT-FLEA SURVEY OF THE MYSORE STATE

BY

P V SEETHARAMA IYER, M A,
King Institute Guindy

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INTRODUCTION

A RAT-FLEA survey of the Madras Presidency (King and Pandit, 1931) financed by the Indian Research Fund Association was carried out under the direction of Lieut-Colonel H H King, I M S, and Dr C G Pandit in thirty different representative areas in the Madras Presidency during 1928–1930 The Indian State of Mysore was not included in the original scheme of work and it was thought that the extension of the survey to Mysore would be of value in making the survey of South India more complete and also of great value to Mysore itself where plague is practically endemic

After consultation with Lieut-Colonel King, Dr Sweet of the Rockefeller Foundation, Consultant in Health to the Mysore Government, asked this Government for sanction for a rapid flea survey of some selected areas in the State It was arranged between Dr Sweet, Dr Karve, the Director of Health, Mysore, and Lieut-Colonel King that the survey would be done by one of the staff of the King Institute, Guindy, under the general supervision of Lieut-Colonel King Extensive surveys were not aimed at but attention was to be paid chiefly to differences in flea species between typically different towns and between typically different areas in the places surveyed

The following six towns were selected and a period of 7–10 days was devoted to each place (*see* Map) —

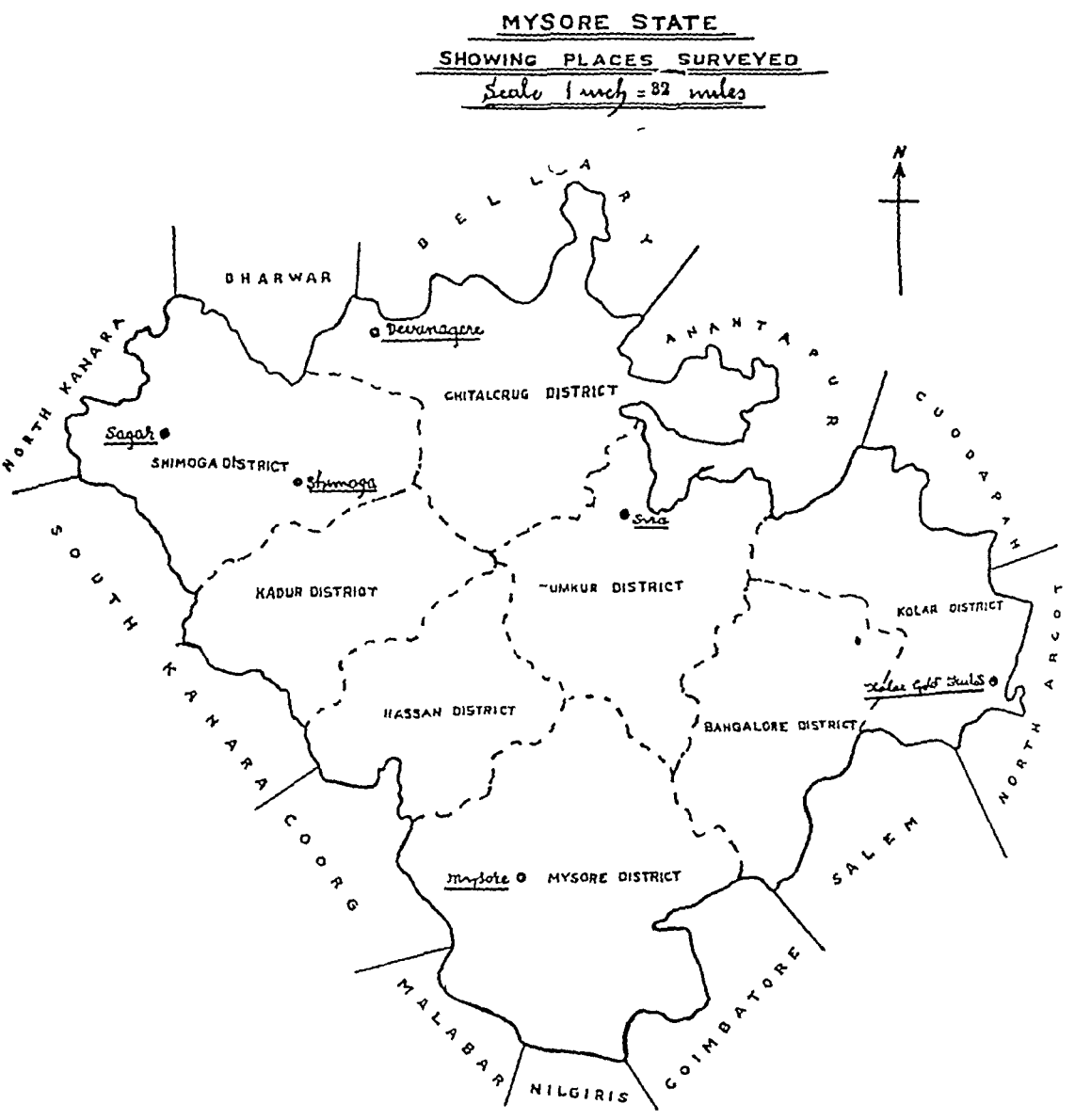
(1) Mysore City
(2) Sira
(3) Davanagere
(4) Sagar
(5) Shimoga
(6) Kolar Gold Fields

GENERAL DESCRIPTION OF MYSORE STATE

The State of Mysore lies on a tableland situated in the angle formed by the Eastern and Western Ghauts It is enclosed by chains of mountains on three

sides, the northern border alone being free It is surrounded by the Madras Presidency on all sides except on part of the west where the Bombay Presidency northwards and Coorg southwards form the boundaries

MAP



The climate of Mysore is on the whole mild and equable owing to its elevation. The rainy months are from June to November, the cold season commences in December and continues till about the end of February March, April and May are the hot months of the year Mysore gets the benefit of both the monsoons, the

South-West commencing in June and the North-East in October The average annual rainfall varies considerably from place to place In order to get an idea of the temperature conditions prevailing during the period of survey, a maximum and minimum thermometer and a wet and dry bulb thermometer were read daily at 8 A M The readings are given in Table I

TABLE I

Meteorological observations

Place surveyed	Period of survey	Temperature					Humidity			Rainfall in inches during survey	Average annual rainfall
		Maximum		Minimum		Mean range	Mean dry bulb temperature	Mean wet bulb temperature	Mean humidity Per cent		
		Mean °F	Highest °F	Mean °F	Lowest °F						
Mysore	5-3-32 to 14-3-32	91 48	92 4	65 4	64 7	26 08	73 75	66 0	63 3		28 9
Sira	16-3-32 to 21-3-32	89 8	92 0	81 6	80 0	8 2	83 25	70 58	49 0		18 13
Davanagere	22-3-32 to 30-3-32	95 8	97 0	76 06	74 5	21 74	77 1	70 2	67 3		24 0
Sagar	1-4-32 to 10-4-32	89 8	93 0	72 2	69 0	17 6	74 77	71 08	80 3	1 2 inches	100 0
Shimoga	11-4-32 to 18-4-32	95 3	97 0	75 07	71 5	20 23	76 36	71 56	76 0	0 95 „	31 8
Kolar Gold Fields	19-4-32 to 28-4-32	86 17	90 0	76 67	73 0	9 5	77 56	71 39	69 8	2 34 „	29 0

A good deal of the internal trade of the State is carried on in the weekly and other periodical 'shandies' (markets) which are held in almost every important place The land-locked position of Mysore and the mountain barriers on the three sides formerly largely prevented active trade with districts outside the State Good roads have recently come into existence and railways have also been extended to

rural districts Buses, country-carts and lorries transport commodities over the Ghauts and there is a brisk trade going on with the bordering districts at present

Buildings

All types of buildings were seen in the places visited The congested localities occupied mostly by the poor class people have thatched or tiled houses with mud walls Ordinary country tiles are supported on a framework of bamboos held in place by wooden beams The tiles usually afford excellent shelter for rats The floors are generally of mud The majority of the houses are dark and ill ventilated Most houses in streets are contiguous affording free scope for rat movement

The rich and the middle classes live mostly in tiled houses with walls made of brick In all the towns surveyed new building is going on consisting of houses built of masonry and bungalows separated from one another by open spaces These belong mostly to the well-to-do classes and have been built on modern sanitary lines They have paved floors and do not afford much shelter for rats

Table II

The number of deaths registered from plague, 1922–1931

Place	Population	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931
Mysore City	107,142	37	321	29	6	48	130	34	77	192	109
Sira	6,894					1					
Davanagere	23,155	2	2	155	60	56	7	2	111		5
Sagar	5,696	18				13					1
Shimoga	20,661	56	35	4	6	26		6	20	18	3
Kolar Gold Fields	85,103	7	1				1	1		8	51

Plague statistics

Table II shows the number of deaths from plague in the various places surveyed for the years 1922–1931 Plague has been prevalent in all these places in an epidemic form at some time or other since 1898 when plague was introduced from the

Bombay Presidency Mysore City, Davanagere and Shimoga are the worst affected and show a fairly high incidence for the whole period Sira alone has escaped from plague—the one death recorded in 1926 was probably an imported case

NOTES ON PLACES SURVEYED

(1) *Mysore City* is the capital of the State and has an area of 9 5 square miles It is 2,525 feet above sea-level It is hotter than Bangalore and exhibits greater extremes of temperature The mean annual 8 A M temperature is 77°F It gets the benefit of both the monsoons, the South-West commencing in June and the North-East in October The chief exports are rice, ragi, tobacco and sandal-wood The grains mainly go to the West Coast, Coimbatore and Nilgiris The imports are mainly piece-goods from Bombay and cotton from Davanagere

(2) *Sira* is a small town in the Tumkur district bordering on Anantapur district of the Madras Presidency The town is 2,223 feet above sea-level The place is a famine area and the town is almost in ruins The soil is hard and poor requiring much labour to render it productive The low altitude and the nature of the soil exert considerable influence on its temperature which resembles that of Bellary and Anantapur districts The mean annual temperature is 79°F The rainfall at Sira is the lowest among the places surveyed, being only 18 13 inches per year (1921–1931) Coco-nut and sugarcane are grown, the dried kernel of the former being exported Coarse woollen blankets are made here and sent out to Bangalore

(3) *Davanagere* is an important and populous trade centre for cotton in the Chitaldrug district The altitude is 1,927 feet above sea-level It adjoins the Bellary district and is drier and hotter than the other parts of Mysore, owing to its lower elevation The mean average annual temperature is 78°F, the highest monthly average being recorded in April and May This place has an annual rainfall of nearly 24 inches, half of this falling between August and November The chief crops grown are cotton, chola and ragi For the last 10 years the trade in ground-nuts has developed to a great extent The railway station goods shed is contiguous to the town and the railway has a special grain siding behind the market to facilitate loading and unloading of goods Wheat and rice for local consumption are imported from outside The chief export consists of cotton to Coimbatore, Dharwar, Bombay and Mysore Scattered on the fringes of the town are a number of cotton presses and ginning factories

(4) *Sagar* is a small town in the Shimoga district and lies to the north-west of Shimoga town on the Shimoga-Gerosoppa road It lies in the heart of the 'Malnad' (hill country) resting on the Western Ghauts The town is 1,960 feet above sea-level The whole of this taluq is studded with magnificent hills and forests affording the most charming scenery The proximity of forests to Sagar exerts

some influence on the climate of this place by affecting the humidity Temperature records are not available The maximum temperature recorded during the survey was 93°F The average annual rainfall is nearly 100 inches, more than 70 per cent of this falling during the South-West monsoon months A few showers also occur in April Sagar is the chief depôt of the areca-nut trade in the State Some sugarcane is also grown

(5) *Shimoga* is the head-quarters of the Shimoga district and lies on the left bank of the river Tunga It is nearly 40 miles from the Western Ghauts and lies at an altitude of 1,899 feet above sea-level The maximum temperature recorded during the survey was 97°F Being farther away from the Western Ghauts than Sagar it has a much smaller rainfall, the average being 31 8 inches Ragi, sugarcane and paddy are cultivated here There are a few godowns in the town stocking paddy and areca-nut

(6) *Kolar Gold Fields* lie to the south of Bowringpet taluq in the Kolar district This district occupies that portion of the Mysore plateau bordering on the Eastern Ghauts What was a wilderness years ago is now a crowded industrial colony comprising an area of nearly 35 square miles The height of the place above sea-level is 2,900 feet

Robertsonpet—a small town in this mining area—was selected for the purpose of this survey The annual means of maximum and minimum temperature at the Kolar Gold Fields are 83°F and 67°F, giving a general average of 75°F The mining area is too far inland to receive much benefit from the South-West monsoon The average annual rainfall is 29 3 inches December to April are usually rainless months The important industry here is, of course, gold mining There are a few oil mills scattered over the area The chief exports are ground-nuts, jaggery and tamarind

Details of survey

The survey commenced on the 4th March, 1932, and was continued till the end of April The month of March is the beginning of the summer season in the State By April hot weather conditions had set in and this might have influenced the prevalence of fleas on rats to some extent

The procedure adopted for the collection and examination of rats and fleas was the same as that described in the report (No 1) on the rat-flea survey of the Madras Presidency (King, Iyer, Natarajan, and George, 1929) Separate data for bazaars and residential areas were always obtained

Rodents

The data regarding the rodents caught during the survey are given in Table III In all 672 rodents were trapped, of which 656 were rats, 5 bandicoots and 11 mice Rodents were caught in 230 traps out of the 2,098 traps distributed

TABLE III

Species and sex of rats

Place	Traps laid	Traps with rats	*Rattus rattus*		*R rattus rufescens*		*R rattus wroughtoni*		Percentage of female *rattus* to total *rattus*	Bandicoots	Mice
			Total	Females	Total	Females	Total	Females			
Mysore	235	41	*111	66	103	63	7	3	59 46		
Sira	260	24	65	31	47	21	18	10	47 70	1	1
Davanagere	374	55	203	112	180	96	23	16	55 20		
Sagar	413	24	42	24	24	13	18	11	57 14	2	
Shimoga	360	35	96	60	34	19	*62	41	62 50	1	
Kolar Gold Fields	456	51	140	68	121	58	19	10	48 60	1	10

* Includes special rats mentioned below

SPECIES AND SEX OF RATS (Table III)

The rats trapped belonged to the species *Rattus rattus* Four varieties were observed (1) *R rattus rufescens*—the common brown-bellied variety chiefly found inside houses (2) *R rattus wroughtoni*—the white-bellied form usually leading an outdoor life but frequently coming into houses at night (3) A lemon-coloured variety, perhaps a colour variation of *R rattus wroughtoni*—this was found only in Shimoga Four of these were obtained These had transparent ears and two pairs of pectoral and three pairs of inguinal mammæ It has been recorded that rats feeding on fruits of prickly pear developed this colour change on the belly fur, but this seems hardly credible (4) A variety with black fur all over the body, of which a single specimen was obtained at Mysore

In Mysore, an urban area and Davanagere and Kolar Gold Fields—both great industrial centres—the percentage of *R rattus wroughtoni* to total *R rattus* was very low, being only 6, 10 and 14 respectively In striking contrast are Shimoga, Sagar and Sira which have extensive open areas all round the town In Shimoga *R rattus wroughtoni* is the predominating variety (65 per cent), while at Sagar and Sira this variety accounts for 43 and 28 per cent respectively

As regards sex, females were in slight excess, forming 55 per cent of the total The lowest percentage of females was at Sira (47 7 per cent) and the highest at Shimoga (62 5 per cent) This excess of females was particularly noticed in the white-bellied *wroughtoni* species where the percentage of females was as high as 62

TABLE IV

Rat density (number of rats per 100 traps laid)

Place	Bazaar	Godowns, cotton mills, etc	Residential area	Whole area
Mysore	190 9	69 19	13 9	47 23
Sira	51 4		11 5	25 0
Davanagere	73 6	12 7	10 5	54 3
Sagar	12 8		8 0	10 2
Shimoga	39 8	6 21	40 7	26 7
Kolar Gold Fields	52 9		17 25	30 7
AVERAGES FOR STATE	43 5	49 3	19 2	31 3

RAT DENSITY (Table IV)

As seen, this general density varied a great deal, it was highest in Mysore In most places the density of rats was higher in the bazaar than in the residential area At Shimoga alone the rat density was about the same in the two areas In Mysore, Sira, Sagar and the Kolar Gold Fields the residential areas yielded only a small proportion of rats, the density ranging from 8 to 18 At Davanagere and Shimoga, however, the figure was higher, 41 rats being caught in each place for every 100 traps set

TABLE V

Pregnancy and replenishment rate

Place	Percentage of pregnant rats to total ♀ rats	Percentage of pregnant rats to total rats	Average number of fœtuses	Replenishment rate for 100 rats per day
Mysore	33 3	19 8	6 54	13 6
Sira	26 0	12 3	5 1	3 92
Davanagere	28 6	15 8	4 9	4 84
Sagar	25 0	14 3	5 16	4 61
Shimoga	38 3	23 96	5 0	7 49
Kolar Gold Fields	33 8	16 4	5 0	5 12

Pregnancy and replenishment rate (Table V)

The percentage of pregnant females to total female rats varied from 25 to 38 3 The average number of fœtuses in pregnant females was fairly uniform in all the areas (4 9 to 5 1), except in Mysore City where it was nearly 6 5 The replenishment rate for 100 rats per day varied from 4 in Sira to as high as 13 6 in Mysore

Rat-flea survey

Four thousand five hundred and seventy-seven fleas were collected on *R rattus* during the survey All the three species of *Xenopsylla* found on rats in South India were present Two specimens of *Ctenocephalus* were also obtained on rats, one at Sira and the other at Kolar Gold Fields *X braziliensis* formed 51 per cent of the total fleas and so is the predominant species in this State The proportions of the three different species varied considerably from place to place

The bandicoots harboured more fleas than *R rattus* and the average number of fleas per bandicoot was nearly 53 It is interesting to note that of the fleas on bandicoots as much as 88 per cent were *X astia,* 9 per cent *X cheopis* and only 3 per cent *X braziliensis*

Table VI

Sex proportions

Name of place	Percentage of female fleas to total fleas	Percentage of female *X astia* to total *X astia*	Percentage of female *X braziliensis* to total *X braziliensis*	Percentage of female *X cheopis* to total *X cheopis*
Mysore	38 6	53 7	33 7	45 4
Sira	44 7	56 6		38 6
Davanagere	30 9	54 3	26 1	27 7
Sagar	36 3	56 7	35 1	33 3
Shimoga	38 85	62 12	38 13	32 46
Kolar Gold Fields	44 2	61 11	39 5	39 6
Whole State	38 7	57 1	33 4	36 7

Sex proportions (Table VI)

As seen, from 31 per cent to 45 per cent—average 38 7 per cent—of fleas were females

While the females of *X cheopis* and *X brazilhensis* formed only 36 7 and 33 4 per cent of their total, they formed as much as 57 1 per cent in the case of *X astia* This definite feature—a high proportion of males among *X cheopis* and *X brazilhensis* and of females among *X astia*—is also brought out when the figures for individual areas are examined separately This observation is in accord with the findings reported in the individual reports of the rat-flea survey of the Madras Presidency

Distribution of fleas and flea indices

The relative prevalence of the three species mentioned differed greatly in the places surveyed Thus, at Sira *X brazilhensis* was entirely absent, while at Kolar Gold Fields this species was absent in the Robertsonpet residential area and in the cooly lines and present only in small numbers in the bazaar, further marked variations in the proportions of the different species of fleas were noticed between different parts of the same town So, the flea population of each place will be considered separately

Table VII

Mysore City

Area	General flea index	*X astia* index	*X brazilhensis* index	*X cheopis* index	Percentage of *X astia* to total fleas	Percentage of *X brazilhensis* to total fleas	Percentage of *X cheopis* to total fleas	Percentage of *R rattus* without fleas	Percentage of *R rattus* without *X astia*	Percentage of *R rattus* without *X brazilhensis*	Percentage of *R rattus* without *X cheopis*
Bazaar	8 27	0 98	5 01	2 27	11 9	60 7	27 4	0	50 8	4 8	11 1
Godowns	14 28	0 52	10 04	3 72	3 6	70 3	26 1	4 0	44 0	4 0	4 0
Residential area	6 74	0 22	3 96	2 57	3 2	58 7	38 1	8 7	82 6	13 0	21 7
Whole area	9 3	0 72	5 93	2 66	7 74	63 7	28 6	2 7	55 9	6 3	11 7

Mysore City

All the three species of *Xenopsylla* were present *X brazilhensis* predominated, forming 64 per cent, *X cheopis* 28 per cent and *X astia* 8 per cent The percentage of *cheopis* to total fleas was a little higher in the residential area than in the bazaar, while the percentage of *astia* was higher in the bazaar

The godowns in Mysore gave a fairly high flea index (14 28) and this was mainly due to the higher proportion of *X brazilhensis* found there The general and specific

indices for the residences were fairly low, the *astia* and the *braziliensis* indices being lower than that of the bazaar and godowns

The index for *cheopis* was fairly uniform The higher index for *X braziliensis* in the godowns is some evidence of the introduction of this species occurring through exchange of commodities

In the bazaar all the rats were flea infested while in the residences nearly 9 per cent of the rats were devoid of fleas A very high percentage of rats (44–83 per cent) were without *X astia* on them, the residential area being particularly marked in this respect On the other hand as seen from the table, there were very few rats without *cheopis* or *braziliensis* except in the residential area This emphasizes the fact that if *X astia* plays any part at all in the transmission of plague in nature in places like Mysore it is certainly very small indeed—figures for plague are in Table II It also follows that plague is almost certainly carried by one or both of the other two species *cheopis* and *braziliensis*

TABLE VIII

Sira

Area	General flea index	*X astia* index	*X cheopis* index	Percentage of *X astia* to total fleas	Percentage of *X cheopis* to total fleas	Percentage of *R rattus* without fleas	Percentage of *R rattus* without *X astia*	Percentage of *R rattus* without *X cheopis*
Bazaar	9 66	2 08	7 55	21 5	78 2	0	10 5	0
Residential area	18 56	8 0	10 56	43 1	56 9	3 7	7 4	3 7
Whole area	13 35	4 54	8 88	34 0	65 9	1 5	9 23	1 5

Sira

The most important feature in this place was the complete absence of *X braziliensis* The general flea index (13 35) was the highest among the places surveyed Another interesting feature was the unusually high flea index (18 56) for the residences with a corresponding high index for the two species of fleas present—*X astia* and *X cheopis*

Seventy-eight per cent of the total fleas in the bazaar were *X cheopis* The proportion of *cheopis* in the residential area was a little lower, only 57 per cent

Very few rats (only 1 5 per cent) were without *cheopis* fleas The percentage of rats without *X astia* was 10 5 in the bazaar and 7 4 in the residential area

The last attack of plague at Sira was ten years ago The town has been free ever since Sira taluq lies adjacent to the Anantapur district of the Madras Presidency and is 33 *miles away from the nearest railway station* This isolation and the comparative absence of trade and other activity probably accounts for the absence of plague in the last ten years But since the climatic conditions in the months of October to November are suitable for the propagation of plague and since *X cheopis* is very numerous, *Sira is likely to suffer severely should it get infected*

TABLE IX

Davanagere

Area	General flea index	*X astia* index	*X brazilensis* index	*X cheopis* index	Percentage of *X astia* to total fleas	Percentage of *X brazilensis* to total fleas	Percentage of *X cheopis* to total fleas	Percentage of *R rattus* without fleas	Percentage of *R rattus* without *X astia*	Percentage of *R rattus* without *X brazilensis*	Percentage of *R rattus* without *X cheopis*
Bazaar	4 69	0 71	2 4	1 62	15 1	50 3	34 6	0 92	53 2	18 3	9 2
Cotton mills and godowns	6 59	0 53	1 83	4 04	8 1	27 7	64 2	0	76 6	34 0	10 6
Residential area	3 34	0 81	1 2	1 36	24 2	35 0	40 8	8 51	87 2	36 2	12 8
Whole area	4 84	0 69	1 97	2 18	14 3	40 7	45 0	2 46	66 5	26 1	10 3

Davanagere

This town being an important cotton centre, separate data were obtained for cotton godowns and ginning mills

The general flea index for the whole town was 4 84 while the index for the cotton godowns and mills was 6 59 The *astia* index was below 1 in all the localities but was the lowest (0 53) in the cotton godowns The *brazilensis* and *cheopis* indices were almost the same in the residential area, while in the bazaar the *brazilensis* index was nearly double that of the residential area The index for *cheopis* in the cotton mills was as usual high, being nearly three times that of the residences As was first reported in the South Indian survey reports by King and Pandit cotton seems to offer exceptional good conditions for the existence and multiplication of *X cheopis* This is borne out by the figures obtained at Davanagere.

Sixty-four per cent of all fleas in the cotton godowns and mills were *X cheopis*, as compared with 34 6 and 40 8 in the bazaar and residences respectively More than half the fleas from the bazaar were *X braziliensis* (50 3 per cent), whereas the proportions elsewhere were only 27 and 35 per cent This higher proportion in the bazaar area again explains the association of this flea with trade

Regarding prevalence of fleas on rats, it was interesting to observe that all the rats from cotton mills and godowns harboured fleas and that the percentage of rats without fleas in the bazaars was less than one *Astia*-free rats were very common indeed, the variations ranging from 53 2 per cent in the bazaar to 87 2 per cent in the residential area *X braziliensis* was absent in 36 per cent of the rats from houses, i e , nearly double that of the bazaar As remarked for Mysore these figures show how small a part if any is played by *X astia* in conveying plague—(figures for plague in Table II) The figures also suggest that plague is carried by one or both of the other two species and, if both, that *cheopis* from its wider prevalence (as seen from the last column of the Table) is the more important in this particular locality, which is perhaps due to its being favoured by the local cotton trade

TABLE X.

Sagar

Area	General flea index	*X astia* index	*X braziliensis* index	*X cheopis* index	Percentage of *X astia* to total fleas	Percentage of *X braziliensis* to total fleas	Percentage of *X cheopis* to total fleas	Percentage of *R rattus* without fleas	Percentage of *R rattus* without *X astia*	Percentage of *R rattus* without *X braziliensis*	Percentage of *R rattus* without *X cheopis*
Bazaar	9 83	0 792	6 25	2 8	8 05	63 14	28 4	0	54 2	4 2	20 8
Residential area	8 78	0 611	6 72	1 44	6 96	76 58	16 46	0	44 4	5 6	27 7
Whole area	9 38	0 714	6 45	2 21	7 61	68 78	23 61	0	50 0	4 8	23 8

Sagar

The general flea index as also the indices for *X astia* and *X braziliensis* were fairly uniform in the different parts of the town The flea index was high, mainly due to the higher proportion of *X braziliensis* on rats The *braziliensis* index was nearly *three* times that of *X cheopis* and *nine* times that of *X astia* The *cheopis* index of the residential area was only half that of the bazaar.

X brazilhensis formed the majority of the fleas—69 per cent The proportion of this species in the different localities was also high and the fairly uniform index for both the bazaar and the residential area is suggestive of wide prevalence

All the rats caught in this place harboured fleas A high proportion of the rats (50 per cent), however, did not have *X astia* on them Very few rats (4 8 per cent) were without *X brazilhensis*, while rats without *X cheopis* were nearly 23 8 per cent of the whole

The comparatively high *brazilhensis* index in the different parts of the town, the higher proportion of this particular species among the fleas collected and the small number of rats not harbouring *X brazilhensis*, all suggest that this species is well established in this town

TABLE XI

Shimoga

Area	General flea index	*X astia* index	*X brazilhensis* index	*X cheopis* index	Percentage of *X astia* to total fleas	Percentage of *X brazilhensis* to total fleas	Percentage of *X cheopis* to total fleas	Percentage of *R rattus* without fleas	Percentage of *R rattus* without *X astia*	Percentage of *R rattus* without *X brazilhensis*	Percentage of *R rattus* without *X cheopis*
Bazaar and godowns	6 83	0 58	4 56	1 7	8 45	66 76	24 79	1 92	59 61	11 54	23 08
Residential area	7 73	0 82	4 57	2 34	10 6	59 12	30 28	0	45 45	22 72	11 36
Whole area	7 24	0 69	4 56	2 0	9 5	63 02	27 48	1 04	53 12	16 67	17 71

Shimoga

The flea index was nearly the same for the bazaar and the residences, and fairly high The *astia* index was below 1, while that of *cheopis* was more than double that of *astia* The index for *brazilhensis* was 4 56 and the high flea index (7 24) was mainly due to this species The number of *cheopis* per rat was higher in the residential area than in the bazaar

As at Sagar, the proportion of *brazilhensis* to total fleas was very high, nearly 63 per cent of the fleas belonging to this species The percentage of *cheopis* to total fleas in the residences was also higher than that of the bazaar

Most of the rats in the town had fleas on them (99 per cent) The percentage of rats without *X astia* was, however, high (53 12)

The figures obtained here suggest that *X brazilhensis* and *X cheopis*, particularly the former, have an even and widespread distribution in this town, and that one or both species, and not *astia* are responsible for carrying plague

TABLE XII

Kolar Gold Fields

Area	General flea index	*X astia* index	*X brazilhensis* index	*X cheopis* index	Percentage of *X astia* to total fleas	Percentage of *X brazilhensis* to total fleas	Percentage of *X cheopis* to total fleas	Percentage of *R rattus* without fleas	Percentage of *R rattus* without *X astia*	Percentage of *R rattus* without *X brazilhensis*	Percentage of *R rattus* without *X cheopis*
Bazaar	3 41	0 47	0 42	2 51	13 87	12 26	73 87	1 4	64 83	72 52	16 48
Robertsonpet residential area	7 0	1 17	0	5 83	16 8	0	83 2	12 2	46 34	100	12 2
Cooly lines	3 62	3 15	0	0 46	93 02	0	6 98	12 5	25 0	100	62 5
Whole area	4 35	0 9	0 271	3 17	20 7	6 24	72 91	7 14	57 14	82 14	17 86

Kolar Gold Fields

The flea indices in this place differed markedly in the different localities surveyed While the general flea index for the whole area was only 4 35, the rats from houses in Robertsonpet had an index of 7 *X brazilhensis* was practically absent in this mining area, an index of only 0 27 being obtained for the whole town This species was present only in the bazaar and was totally absent on rats from the cooly lines and houses in Robertsonpet The absence of this species from areas such as this where bazaar and trade conditions are at a minimum again emphasizes its association with these conditions Its presence in the bazaar only suggests its gradual introduction

Regarding *cheopis*, while the index for this species at Robertsonpet was fairly high, it was below 1 in the cooly lines The sanitation in the cooly lines is under the control of the mining authorities and a rat gang is continuously working throughout the year At the different mines in the Kolar Gold Fields there are gangs of ratcatchers working under the supervision of sanitary overseers Rodents are, not

trapped but are dug out of burrows and killed The burrows are sometimes extensive and the labour involved in tracing up a burrow is arduous but once the end of the burrow is reached, a whole family of rodents can be exterminated Compared with its expense the value of this method is doubtful, for in a large proportion of the burrows only mice are found The cooly lines mainly consist of huts having a single room with a verandah outside The roof is of zinc sheets and the sanitary condition of the lines is better than that of Robertsonpet At the instance of the Health Officer, Kolar Gold Fields, a few rats were trapped in Block I where plague first starts almost every year The fleas found on these rats were mainly *X cheopis*

In the cooly lines only 7 per cent of the fleas were *cheopis*, while 93 per cent were *X astia* The position was reversed in Robertsonpet where 83 per cent of the fleas on rats were *X cheopis* Again, in the cooly lines as much as 62 5 per cent of the rats were without *X cheopis*, while in Robertsonpet the percentage of rats without *X cheopis* was much less—12 2 per cent Thus, the interesting features regarding fleas in this place were —

1 The absence of *X brazilhensis* in all places except the bazaar where a few were obtained suggesting recent introduction

2 The greater number of *cheopis* on rats in Robertsonpet as compared with the cooly lines—improved sanitation and organized rat destruction by the opening of burrows are probably contributory causes

3 A higher *cheopis* index for rats caught in an area where it is reported that plague usually starts

TABLE XIII

Comparison of places surveyed

Name of place	Altitude (in feet above sea level)	Average annual rainfall	Flea index	*X astia* index	*X brazilhensis* index	*X cheopis* index	Number of deaths from plague per year per 1,000 of population (1922-1931)
Davanagere	1,927	24 0	4 84	0 69	1 97	2 178	17 4
Mysore	2,525	28 9	9 3	0 72	5 93	2 66	9 2
Shimoga	1,899	31 8	7 24	0 69	4 56	2 0	8 3
Sagar .	1,960	100 0	9 38	0 71	6 15	2 21	5 2
Kolar Gold Fields.	2,900	29 0	4 35	0 9	0 27	3 17	0 81
Sira ..	2,223	18 13	13 35	4 54		8 88	0 14

COMPARISON OF PLACES SURVEYED (Table XIII)

The first striking fact of the comparison given in the table is the relatively very high flea indices for *astia* and *cheopis* in Sira with the comparative absence of plague which is accounted for by the special local conditions The next striking fact is that, apart from this exception, while the indices for *astia* and *cheopis* vary very little from place to place, the index for *braziliensis* varies a great deal This is so striking that we are tempted to conclude that there is some special factor or set of factors that affects this species alone One of these factors may be climate, particularly humidity, for it was most abundant in Sagar which has the highest rainfall, and least abundant—absent—in Sira, with the lowest rainfall But it is doubtful whether this can be the main factor, for the climate of Sira was good enough from a general rat-flea point of view to give the highest general flea index, further the differences between the other towns do not correspond with climate One possible factor—the grain trade—will be discussed in the next section

The column for plague figures does not bring out any striking fact, but it is to be noted that the relatively high incidence of plague in Davanagere may be associated with its being a *cotton* centre and thus exposed to more importation of *adult* fleas which alone can carry infection The lessened importation of infection possibly explains the small figure for plague in Kolar and Sira, for these two towns compared with the others are conspicuous for the comparative absence of the grain and cotton trades

It is difficult to draw any certain conclusions as regards the association of flea species with plague The first four towns have suffered moderately severely from plague, the last two very little The *X astia* index is practically uniform except for the high figure in the last town The small index for *astia* and as noted under Mysore, Davanagere and Shimoga, the high figure for the percentage of rats free from this species make it *almost certain that* X astia *plays practically no part in the transmission of plague in Mysore State* The *cheopis* index is roughly the same in the first four and is higher in the last two As noted for the three towns that mostly suffered from plague—Mysore, Davanagere and Shimoga—, *cheopis* is very well distributed, so that, although the index is not high, it almost certainly plays its part in the dissemination of plague The *braziliensis* index is definitely higher in the first four and is low or *nil* in the last two , further as noted in the local reports it is well distributed So apart from the comparatively low index for *braziliensis* for the first town (Davanagere) which suffered most heavily from plague—which we have associated with *cheopis* and the cotton trade there—, Table XIII as a whole suggests the association of *X braziliensis* with the transmission of plague in nature, but no certain conclusion can be drawn from these figures since *cheopis*—a known carrier—is also present As found by Webster and Chitre (1929) in rat pit experiments *X braziliensis* carries plague very easily

TABLE XIV

Flea index for Mysore State

Nature of area	FLEA INDEX			*X astia* INDEX			*X braziliensis* INDEX			*X cheopis* INDEX		
	Highest	Lowest	Average	Highest	Lowest	Average	Highest	Lowest	Average	Highest	Lowest	Average
Residences	18 56	2 14	7 74	8 0	0 22	**1·87**	6 72	0	2 25	10 56	1 36	3 62
Bazaar	9 83	3 41	6 1	2 08	0 47	0 82	6 25	0	2 65	7 55	1 62	2 63
Godowns	14 3		14 3	0 52		0 52	10 04		**10·04**	3 72		3 72
Cotton mills and cotton godowns	6 59		6 6	0 53		0 53	1 83		1 83	4 23		**4·23**
Whole area			6 97			1 12			2 74			3 1

COMPARISON OF LOCALITIES (Table XIV)

General flea index —The figures show, firstly, how much more abundant rat-fleas are in godowns than elsewhere and, secondly, that they were as abundant, in fact, even a little more abundant, in ordinary houses and huts than in bazaar areas

X astia —As seen, the index is low, but is definitely higher in residential huts and houses as compared with bazaars, etc , i e , it is common in the periphery This, with its being the commonest flea on field rats and bandicoots (*see* above) confirms King and Pandit's conclusion as to its probably being an indigenous flea

X braziliensis —A most striking fact is its abundance in godowns, which were almost all grain godowns, and its relative scarcity in cotton mills and cotton godowns It seems to be definitely associated with the grain trade It being the only species, whose index in bazaar areas was higher than in residences, is in line with this It is probably the main factor which was being searched for in the previous section to account for the differences between the surveyed towns as regards its prevalence Thus, Sira and Kolar Gold Fields with the lowest *braziliensis* indices have not got either the grain or cotton trade The absence of the cotton trade cannot be the

explanation, for, as seen from the table, this species was relatively least abundant in cotton mills and godowns Thus, the last two tables definitely suggest a special close association between *X braziliensis* and the grain trade

Conclusions

(1) The white-bellied *R rattus wroughtoni* was common and in some cases predominated over the ordinary brown-bellied (*rufescens*) variety

(2) As regards sex, females were in slight excess forming 55 per cent of the total rats This excess was particularly marked in the white-bellied variety where the percentage of females was as high as 62

(3) The three common South Indian species of *Xenopsylla* were found Their relative prevalence is best shown by the specific indices for the whole area —

X astia	1 12
X braziliensis	2 74
X cheopis	3 1

(4) While the females of *X cheopis* and *braziliensis* formed only 36 and 33 per cent of the total, they formed as much as 57 per cent in the case of *X astia* suggesting a more rapid leaving of their host for egg laying in the case of the former two species and so possibly better breeding power

(5) The species commonest in the following localities were as follows —

(*i*) On field rats and bandicoots—*X astia* 88 per cent
(*ii*) Residences—*X cheopis* index 3 62 forming 47 per cent of the total
(*iii*) Bazaars—*X braziliensis* index 2 6 forming 43 per cent of the total
(*iv*) Godowns (mainly grain)—*X braziliensis* index 10 forming 70 per cent of the total
(*v*) Cotton mills and godowns—*X cheopis* index 4 2 forming 64 per cent of the total

(6) Certain facts strongly suggest the association of *X cheopis* with the cotton trade and of *X braziliensis* with the grain trade, and with these two trades, it is known, and there is also evidence here, that plague is associated

(7) While, as known, the transmission of plague is certainly associated with *X cheopis*, certain facts suggest that here it is also associated with *X braziliensis* On the other hand, there is definite evidence that the transmission of plague is not associated with *X astia*

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THE INFLUENCE OF BLOOD GROUP IN CERTAIN PATHOLOGICAL STATES

BY

CAPTAIN P N MITRA, M B, D T M,

Teacher of Pathology, B W M School, Dibrugarh

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THE writer recently made an attempt to find out if blood group has any influence on the incidence of disease and worked out the blood group of 2,000 persons, sick and healthy, the latter to serve as controls The sick included consecutive admissions to hospital, of men and women of all castes and creeds, for all sorts of diseases, without any selection

TABLE I

	GROUP A		GROUP B		GROUP AB		GROUP O		TOTAL
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	
Sick only	349	23 67	482	32 7	146	9 9	497	33 71	1,474
Healthy controls only	142	26 99	169	32 12	39	7 41	176	33 46	526
Sick and healthy both	491	24 55	651	32 55	185	9 25	673	33 65	2,000

Table I shows the distribution of the sick and the healthy according to their blood groups Some differences in the percentage distribution are at once seen and the question suggests itself whether such differences represent a differentiated sample of the general population or simply represent chance variation To put it in a different way, what is the probability that the one distribution is really different from the other to a greater degree than could reasonably be supposed to have arisen by the operation of chance alone ?

Pearson (1911-12) shows that if we let the population from which the two samples, if undifferentiated, are supposed to be drawn, be given by the class frequencies m_1, m_2, m_3, m_4 ... m_p, m_q ... m_s

The total population being M, and let the samples be given by the frequencies in the same classes —

							Total
First sample	f_1	f_2	f_3	...	f_p	f_q	... f_s	N
Second sample	f'_1	f'_2	f'_3	...	f'_p	f'_q	... f'_s	N'

Where the totals N and N' differ widely or little, and then form a quantity,

$$x^2 = S_1^s \left\{ \frac{N N' \left(\frac{f_p}{N} - \frac{f'_p}{N'} \right)^2}{f_p + f'_p} \right\}$$

Where S^s denotes summation of like quantities from 1 to s, that then the required probability that the two samples are undifferentiated, i.e., did come as random samples from the same population, may be found by working out the value of P corresponding to the ascertained x^2 and n^1 (the number of classes) from the tables given on pages 26 to 29 of Pearson's 'Tables for Statisticians and Biometricians'

		Group A	Group B	Group AB	Group O		Total
Sick only	(i)	349	182	146	197	f	1,474
Healthy control	(ii)	142	169	39	176	f^1	526
(i) + (ii)	(iii)	491	651	185	673	$f+f^1$	2,000
(i)/1474	(iv)	0 2367	0 3270	0 0990	0 3371	f/N	
(ii)/526	(v)	0 2699	0 3212	0 0741	0 3155	f^1/N^1	
(iv)—(v)	(vi)	0 0332	0 0058	0 0249	0 0216	$f/N - f^1/N^1$	
Square of (vi)	(vii)	0 00100324	0 00003364	0 00062001	0 00046656	$(f/N - f^1/N^1)^2$	
(vii)—(iii)	(viii)	0 0000020	0 00000005	0 0000033	0 00000007	$\frac{(f/N - f^1/N^1)^2}{f+f^1}$	0 00000615

$$x^2 = N N^1 \times 0\ 00000615 = 1{,}474 \times 526 \times 0\ 00000615 = 4\ 76824260 = \text{say } 5$$

If $x^2 = 5$ and $n^1 = 4$ then P according to the tables $= 0\ 171797$ That is to say there are 17 chances in a hundred in favour of the samples being random samples from the same population that is to say the sick, collectively, and the healthy do not differ materially in their blood groups

It appears from the above that if all sorts of sick are taken then there is no distinction in the distribution of blood groups between them and the healthy The sick was then classified according to the diseases and the distribution of each disease

was studied separately Table II shows the percentage distribution of the various diseases according to their blood groups

TABLE II

Diseases	Total number	Group A		Group B		Group AB		Group O		Remarks
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	
Malaria	265	58	21 88	96	36 22	22	8 30	89	33 58	
Other protozoal infections	53	9	16 58	16	30 18	4	7 54	24	45 28	Includes only amœbiasis and leishmaniasis
All protozoal infections	318	67	21 06	112	35 20	26	8 17	113	35 53	
Helminthiasis	112	27	24 10	31	27 67	18	16 07	36	32 14	
Of the digestive system	131	34	25 95	49	37 40	6	4 58	42	32 06	
„ „ skin	127	36	28 34	37	29 13	13	10 23	41	32 28	
„ „ eye	93	21	22 58	31	33 33	12	12 90	29	31 18	
„ „ nervous system	41	8	19 51	13	31 70	7	17 07	13	31 70	
Malignant disease	16	1	6 25	4	25 00	5	31 25	6	37 25	
Syphilis	41	8	19 51	13	31 70	3	7 31	17	41 46	
Leprosy	14	6	42 84	3	21 42	2	14 28	3	21 42	
Tuberculosis	77	15	19 48	28	36 36	7	9 09	27	35 06	
Pneumococcal infections	26	5	19 23	6	23 07	5	19 23	10	38 46	
All bacterial infections	284	70	24 64	96	33 80	28	9 85	90	31 69	
Of the respiratory system	44	8	18 18	15	34 09	1	2 27	20	45 45	Excluding pneumococcal infections

Calculated according to Pearson's formula, in no disease did the affected population appear as differentiated samples from the general population, except in the cases of helminthiasis and malignant disease The last two will therefore be examined in greater detail

		Group A	Group B	Group AB	Group O		Total
Other persons	(i)	464	620	167	637	f	1,888
Helminthiasis cases	(ii)	27	31	18	36	f'	112
(i)+(ii)	(iii)	491	651	185	673	$f+f'$	2,000

Calculating as before the value of $\frac{(f/N-f'/N')^2}{f+f'} = 0\ 00003377$, so $\chi^2 = N\,N' \times 0\ 00003377 = 1,888 \times 112 \times 0\ 00003377 = 7\ 14086912 =$ say 7

According to the tables when $\chi^2=7$ and $n'=4$, $P=0\ 071897$ Or in other words there are only seven chances in a hundred in favour of the samples being undifferentiated ones These are probably differentiated

I shall examine these figures a little more in detail with a view to find out the exact point of difference, with the help of another formula of Pearson (1907)

Let it be supposed that a first sample of $n=p+q$ be drawn from the population, p denoting the number of times the event dealt with occurs in the n trials, and q the number of times it fails

Write $p = \frac{p}{n}$, $q = \frac{q}{n}$

whence, of course, $p+q = 1$

we then have for the chief constants of the error distribution for a second sample of magnitude m drawn from the same population the following values —

Mean $= mp + \frac{m}{n+2}(q-p)$

Mode = the integral portion of $mp+p$

σ = standard deviation $= \left\{ m\left(p + \frac{q-p}{n+2}\right)\left(q - \frac{q-p}{n+2}\right)\left(1 + \frac{m-1}{n+3}\right)\right\}^{\frac{1}{2}}$

Probable error $= P\,E = \pm\ 0\ 67449\ \sigma$

I shall start with group A of the helminthiasis cases

In this case, $n = 1,888$

$$p = 464 = \frac{464}{1,888} = 0\ 2457$$

$$q = 1,424 = \frac{1,424}{1,888} = 0\ 7543$$

$$m = 112$$

$$p-q = 0\ 7543 - 0\ 2457 = 0\ 5086$$

Here mean $= 112 \times 0\,2157 + \frac{112}{1,890} \times 0\,5086 = 27\,5184 + 0\,059 \times 0\,5086$
$= 27\,5184 + 0\,03 = 27\,5181$

Mode $= 27\,5181 + 0\,2157 = 27\,7641$, i e , 27

$$\sigma = \left\{ 112 \left(0\,2157 + \frac{0\,5086}{1,890}\right) \left(0\,7543 - \frac{0\,5086}{1\,890}\right) \left(1 + \frac{111}{1,891}\right) \right\}^{\frac{1}{2}}$$
$$= \left\{ 112\,(0\,2157 + 0\,0002)\,(0\,7543 - 0\,0002) \times 1\,05 \right\}^{\frac{1}{2}}$$
$$= \left\{ 112 \times 0\,2159 \times 0\,7541 \times 1\,05 \right\}^{\frac{1}{2}} = \left\{ 117\,6 \times 0\,2459 \times 0\,7541 \right\}^{\frac{1}{2}}$$
$$= \sqrt{21\,79} = 4\,66$$

$P\,E = \pm\, 0\,67449 \times 4\,66 = \pm\, 3\,14$

Now the actual number of helminthiasis cases in group A is 27 This agrees with the expected number as calculated above

In group B of the helminthiasis cases,

$n = 1,888$
$p = 620 = \frac{620}{1,888} = 0\,3283$
$q = 1,268 = \frac{1,268}{1,888} = 0\,6717$
$m = 112$
$q - p = 0\,6717 - 0\,3283 = 0\,3434$

Mean $= 112 \times 0\,3283 + \frac{112}{1,890} \times 0\,3434 = 36\,7696 + 0\,02 = 36\,8$

σ as calculated above $= 5\,07$

$P\,E = \pm\, 0\,67449 \times 5\,07 = \pm\, 3\,4$

So the mean with its probable error is $36\,8 \pm 3\,4$ The actual number of helminthiasis cases in group B is 31 and agrees with the expected number as calculated above

In group AB of the helminthiasis cases,

$n = 1,888$
$p = 167 = \frac{167}{1,888} = 0\,0884$
$q = 1,721 = \frac{1,721}{1,888} = 0\,9116$
$q - p = 0\,9116 - 0\,0884 = 0\,8232$
$m = 112$

Mean $= 112 \times 0\,0884 + \frac{112}{1,890} \times 0\,8232 = 9\,90 + 0\,048 = 9\,948$

Mode $= 9\,90 + 0\,0884 = 9\,9884$, i e , 9

$$\sigma = \left\{ 112 \left(0\,0884 + \frac{0\,8232}{1,890}\right) \left(0\,9116 - \frac{0\,8232}{1,890}\right) \left(1 + \frac{111}{1,891}\right) \right\}^{\frac{1}{2}}$$

$$= \left\{ 112 \left(0\ 0884 + 0\ 0004\right) \quad \left(0\ 9116 - 0\ 0004\right) \left(\frac{1,891+111}{1,891}\right) \right\}^{\frac{1}{2}}$$

$$= \left\{ 112 \times 0\ 088 \times 0\ 9112 \times 1\ 05 \right\}^{\frac{1}{2}} = \sqrt{1\ 04} = 1\ 02$$

$P\,E = \pm\ 0\ 67449\ \sigma = \pm\ 0\ 67449 \times 1\ 02 = \pm\ 0\ 68$

So the mean with its probable error = 9 948 ± 0 68 = say 10 The actual number of helminthiasis cases in group AB being 18, is certainly much more than expected

In group O of the helminthiasis cases,

$n = 1,888$

$p = 637 = \frac{637}{1,888} = 0\ 3373$

$q = 1,251 = \frac{1,251}{1,888} = 0\ 6627$

$q - p = 0\ 6627 - 0\ 3373 = 0\ 3254$

$m = 112$

Here mean $= 112 \times 0\cdot3373 + \frac{112}{1,890} \times 0\ 3254 = 37\ 789$

$\sigma = 5\ 08$

Probable error $= \pm\ 0\ 67449 \times 5\ 08 = \pm\ 3\ 42$

So the mean with its probable error = 37 789 ± 3 42

The actual number of helminthiasis cases in group O being 36 the agreement is very close

So the distribution of groups A, B and O, among the cases of helminthiasis is normal, but there is a preponderance of persons of group AB

Turning next to the cases of malignant disease we see that

		Group A	Group B	Group AB	Group O		Total	Remarks
Other persons	(i)	490	647	180	667	f	1,984	
Malignant disease	(ii)	1	4	5	6	f'	16	Includes only cancer and sarcoma
(i) + (ii)	(iii)	491	651	185	673	$f+f'$	2,000	

Calculating as before we find that $\frac{(f'N - f'/N')^2}{f+f'} = 0\ 0003461$

$x^2 = NN' \times 0\ 0003461 = 1,984 \times 16 \times 0\ 0003461 = 10\ 9865984 =$ say 11

If $x^2 = 11$ and $n' = 4$ then P according to the tables $= 0\ 011726 =$ say 0 01

That is to say there is only one chance in a hundred in favour of the samples being random samples In other words the probabilities are that they are differentiated samples

I shall now take the different groups of the malignant disease one after another, and begin with group A

Here $n = 1,984$

$p = 490 = \frac{490}{1,984} = 0\ 2469$

$q = 1,494 = \frac{1,494}{1,984} = 0\ 7531$

$q-p = 0\ 5062$

$m = 16$

$$\text{Mean} = 16\times 0\ 2469 + \frac{16}{1,986}\times 0\ 5062 = 3\ 956$$

$$\text{Mode} = 16\times 0\ 2469 + 0\ 2469 = 3\ 9504 + 0\ 2469 = 4\ 1973 = 4$$

$$\sigma = \left\{ 16\left(0\ 2469 + \frac{0\ 5062}{1,986}\right)\left(0\ 7531 - \frac{0\ 5062}{1,986}\right)\left(1 + \frac{15}{1,987}\right)\right\}^{\frac{1}{2}}$$

$$= 1\ 72$$

Probable error $= \pm 0\ 67449 \times 1\ 72 = \pm 1\ 16$

The actual number of cases of malignant disease in this group being only one, is four times less than the most probable expected number, namely four

In group B of the malignant disease,

$n = 1,984$

$p = 647 = \frac{647}{1,984} = 0\ 3261$

$q = 1,337 = \frac{1,337}{1,984} = 0\ 6739$

$q-p = 0\ 6739-0\ 3261 = 0\ 3478$

$m = 16$

$$\text{Here mean} = 16\times 0\ 3261 + \frac{16}{1,986}\times 0\ 3478 = 5\ 212$$

$$\sigma = \left\{ 16\left(0\ 3261 + \frac{0\ 3478}{1,986}\right)\left(0\ 6739 - \frac{0\ 3478}{1,986}\right)\left(1 + \frac{15}{1,987}\right)\right\}^{\frac{1}{2}}$$

$$= \sqrt{3\ 89} = 1\ 97$$

$$PE = \pm 0\ 67449\times 1\ 97 = \pm 1\ 33$$

So the mean with its probable error $= 5\ 212 \pm 1\ 33$ The actual number of cases of malignant disease in this group being four, the agreement is very close

In group AB of the malignant disease,

$$n = 1{,}984$$
$$p = 180 = \frac{180}{1{,}984} = 0\ 0907$$
$$q = 1{,}804 = \frac{1{,}804}{1{,}984} = 0\ 9093$$
$$q-p = 0\ 9093-0\ 0907 = 0\ 8186$$
$$m = 16$$

$$\text{Here mean} = 16\times 0\ 0907 + \frac{16}{1{,}986} \times 0\ 8186 = 1\ 456$$

$$\text{Mode} = 16\times 0\ 907 + 0\ 0907 = 1\ 5407 = 1$$

$$\sigma = \left\{ 16 \left(0\ 0907 + \frac{0\ 8186}{1{,}986}\right)\left(0\ 9093 - \frac{0\ 8186}{1{,}986}\right)\left(1 + \frac{15}{1{,}987}\right)\right\}^{\frac{1}{2}}$$
$$= \sqrt{1\ 44} = 1\ 2$$

$$PE = \pm\ 0\ 67449\times 1\ 2 = \pm\ 0\ 81$$

The actual number of malignant disease cases in this group being five, is five times the modal or the most probable number and over twice the mean with its probable error

In group O of the malignant disease,

$$n = 1{,}984$$
$$p = 667 = \frac{667}{1{,}984} = 0\ 3361$$
$$q = 1{,}317 = \frac{1{,}317}{1{,}984} = 0\ 6639$$
$$q-p = 0\ 6639-0\ 3361 = 0\ 3278$$
$$m = 16$$

$$\text{Here mean} = 16 \times 0\ 3361 + \frac{16}{1{,}986} \times 0\ 3278 = 5\ 38$$

$$\text{Mode} = 16 \times 0\ 3361 + 0\ 3361 = 5\ 7137 = 5$$

$$\sigma = \left\{ 16 \left(0\ 3361 + \frac{0\ 3278}{1{,}986}\right)\left(0\ 6639 - \frac{0\ 3278}{1{,}986}\right)\left(1 + \frac{15}{1{,}987}\right)\right\}^{\frac{1}{2}}$$
$$= \sqrt{3\ 95} = 1\ 99$$

$$PE = \pm\ 0\ 67449\times 1\ 99 = \pm\ 1\ 34$$

Here the actual figure is six in this group and is within range of the mean

So it is seen that, in malignant disease, the distribution of groups B and O is normal, group A is diminished and group AB is increased

The increase in group AB in malignant disease has previously been noted by other observers But Schiff contends that this apparent increase is due to pseudo-agglutination, which occurs in cancer Snyder (1929) also says that pseudo-agglutination occurs in rheumatism, tuberculosis and pneumonia

Tuberculosis

		Group A	Group B	Group AB	Group O		Total
Other persons	(i)	476	623	178	646	f	1,923
Tubercular persons	(ii)	15	28	7	27	f'	77
(i) + (ii)	(iii)	491	651	185	673	$f+f'$	2,000

Here $\frac{(f/N-f'/N)^2}{f+f} = 0\ 00000841$

$x^2 = N N' \times 0\ 00000841 = 1,923 \times 77 \times 0\ 00000841 = 1\ 24527711 =$ say 1

If $x^2 = 1$ and $n' = 4$ then $P = 0\ 801253 =$ say 0 8

That is to say there are eighty chances to a hundred in favour of the two samples being random samples from the same population These are not differentiated samples

Pneumococcal infections

		Group A	Group B	Group AB	Group O		Total
Other cases	(i)	486	645	180	663	f	1,974
Pneumococcal infections	(ii)	5	6	5	10	f'	26
(i) + (ii)	(iii)	491	651	185	673	$f+f'$	2,000

Here $\frac{(f/N-f/N')^2}{f+f'} = 0\ 0000773$

$x^2 = N N' \times 0\ 0000773 = 1,974 \times 26 \times 0\ 0000773 = 3\ 9642532 =$ say 4

If $x^2 = 4$ and $n' = 4$ then $P = 0\ 261464 =$ say 0 3

That is to say there are thirty chances to a hundred in favour of the samples being random samples from the same proportion The samples then are not differentiated samples

So, it is apparent that the distribution of blood groups in tuberculosis and pneumococcal infections is normal Pseudo-agglutination has not affected the

results The technique has been correct There is then no reason to believe that pseudo-agglutination has vitiated my results in malignant disease and helminthiasis

Conclusion —Persons of group AB suffer from an increased liability to malignant disease and helminthic infections Persons of group A are expected to show a diminished liability to malignant disease

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THE ACTION OF QUINAMINE ON THE HEART

BY

J CHRISTODOSS DAVID, M B , B S (Madras), PH D (Edin),

AND

C VAREED, M B , B S (Madras)

(*From the Department of Pharmacology, Madras Medical College*)

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CHOPRA AND DAVID (1927) published the first account of the pharmacological action of quinamine, a comparatively little-known alkaloid of cinchona bark, which was isolated by Hesse

Quinamine is a dextro-rotatory alkaloid and has marked pharmacological actions Chopra and David found that this was the most powerfully acting alkaloid of cinchona on plain muscle like the uterus They found that quinamine had a somewhat weaker action on the circulation than other alkaloids of the cinchona bark, causing a transient fall of blood-pressure, and decreasing both the force and frequency of the cardiac beat The present work was undertaken to elucidate further the action of this drug on the heart with special reference to its possible usefulness in the treatment of auricular fibrillation The work has been rendered possible by the courtesy of G E Shaw, Esq , Quinologist to the Bengal Government, who supplied us with sufficient quantities of the alkaloid, and our thanks are due to him

Experiments were done on the hearts of dogs, cats, and frogs Urethane anæsthesia was used in the case of dogs and cats The results of the experiments are as follows —

On blood-pressure —Quinamine when given intravenously in small doses to experimental animals has only a slightly depressing effect on the blood-pressure

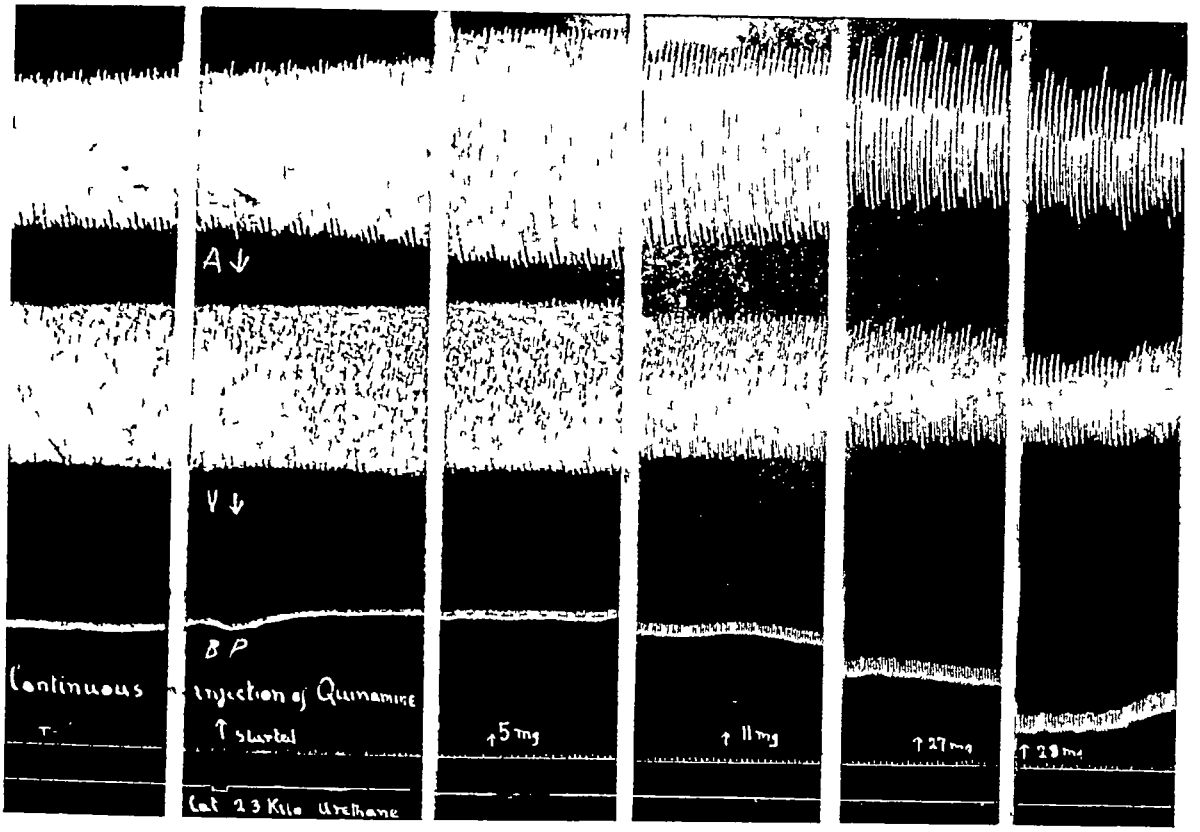
Five milligrams hardly produce any effect in a cat of average size Between 5 and 10 mg , the fall is well marked, but the pressure quickly recovers its original height This fall is in no way affected after section or paralysis of the vagi

Chopra and David (1927) pointed out that quinamine causes a well-marked rise in the volume of the intestines which is even more appreciable in the case of the spleen This dilatation of the splanchnic vessels partly contributes to the fall of blood-pressure A direct depressant action on the heart which is mentioned hereafter is another contributory cause

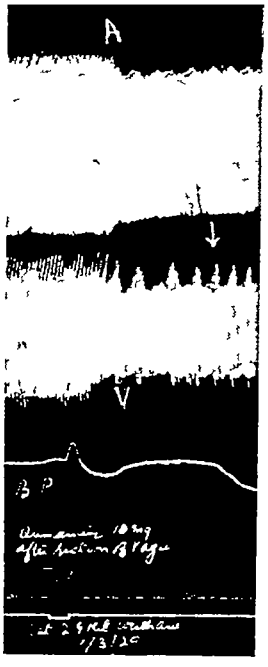
On the heart —Graph 1 shows the effect of quinamine on the auricle and the ventricle of the cat. A continuous injection of the drug was given into the saphenous vein and records of the auricular and ventricular tracings were taken It is noticed that up to 5 milligrams no appreciable fall of blood-pressure occurs but there is a progressive stimulation of the auricular contraction The ventricular contractions are unaltered With larger doses the blood-pressure gradually falls and the transient stimulation of the auricle tends to disappear With much larger doses both the auricular and ventricular contractions are depressed and the fall of blood-pressure is greater The initial stimulation of the auricle might be due to a stimulation of the sympathetic mechanism or a depression of the vagus nerve After doses of ergotoxin which abolished the pressor action of adrenalin, the stimulation of the auricle still occurs with quinamine But this effect is absent both after section of the vagi in the neck (Graph 2, *a*) and also when the vagus nerve-endings in the heart have been paralysed by injections of atropine Quinamine therefore appears to act on the cardio-inhibitory centre in the medulla reducing the tone of the vagus and this is the cause of the initial apparent stimulation of the auricle observed after small doses of quinamine With large doses, the direct depressant action of the drug on the cardiac muscle overshadows the result of vagal depression and then a purely depressant effect is noticed both on the auricle as well as on the ventricle A gradual weakening of the heart-beat, dilatation and final arrest in diastole complete the picture

Perfusion experiments both on mammalian and amphibian heart show that even high dilutions such as 1 in 320,000 cause a diminution of amplitude and weakening of the heart, due to a direct muscular effect Continuous perfusion of the isolated heart of the frog was done with the aid of Clark's cannulæ, one being tied into the sinus venosus , the other leading the perfusion fluid from the heart through the *truncus arteriosus* back to the first perfusion cannula As small a dose as 0 003 mg of quinamine in the perfusion fluid causes an appreciable weakening of the systole and a dilatation of the heart (Graph 2, *c*) Graph 2, *b* shows the depression of the isolated heart of the kitten caused by a dilution of 1 in 250,000 of quinamine

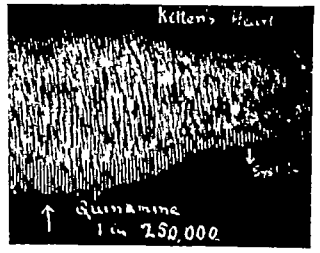
GRAPH 1



GRAPH 2.



a



b

c

The irritability of the cardiac muscle is greatly reduced by quinamine The minimal effective current for stimulating the heart of a frog is found to be considerably increased as shown by the following table —

Distance of secondary from primary coil in centimetres

Number	Before quinamine	Dose in mg	After quinamine
1	18 5	0 25	8 5
2	14 5	0 5	4 0

The same effect was noticed in the case of the dog's heart, but to a smaller degree After an intravenous injection of 10 mg of quinamine, the secondary coil had to be brought nearer by 15 mm , from 15 5 cm to 14 cm

The effect of quinamine on the latent period was studied in frogs A stannius preparation was made by putting a soft clamp over the white crescent and the ventricle was stimulated by minimal induced shocks The normal interval between the moment of stimulation and beginning of contraction was measured After removing the clamp an injection of the drug was given intra-hepatically , the crescent was again clamped, the ventricle stimulated and the interval between stimulation and contraction measured as before Studied according to this method, it was found that quinamine neither increased nor decreased the latent period

The influence of quinamine on the refractory period of the heart was studied in the cat, turtle, and the frog The technique used was that mentioned by Waddell (1924) A straight wire was attached to the armature of a signal magnet and was so arranged as to pull the writing lever away from the drum as soon as the circuit was made The magnet was taken in the primary circuit and the surface of the heart was stimulated by platinum electrodes from the secondary coil Normally, a stimulus applied anywhere in the diastolic phase produces a contraction and a compensatory pause, while the heart is refractory throughout the systolic phase Quinamine does not alter the refractory period in any way It neither decreases nor increases it The same result was obtained in the heart of all three animals— the frog, the turtle and the cat

The absence of any action prolonging the refractory period pointed to the futility of trying this drug in auricular fibrillation Marked irregularity of the auricle was induced in the cat by intravenous injections of aconitine, and quinamine was administered after it The irregularity slightly increased and no favourable effect was noticed on the fibrillations

Discussion —The experiments mentioned above show that quinamine causes an apparent stimulation of the auricle by a depressant action on the vagal centre

in the medulla At the same time a depression of the ventricle occurs by a direct action on the myocardium So far the action is similar to that of the other dextro-rotatory alkaloids—quinidine and cinchonine Possibly in the case of the auricle the nervous factor predominates while in the case of the ventricle the direct toxic action of the drug on the muscle overbalances the nervous effect The vagus supplies the auricle and has an effect on all parts of the auricle, while only the base of the ventricle is supplied by this nerve Thus the control of the vagus over the ventricle is inconsiderable Starling states, ' It is still doubtful whether the vagus has a direct action on the mammalian ventricle The fact that stimulation of the *vagus, like division of the bundle of His, usually causes merely temporary cessation* of the ventricular beat, would indicate that this nerve has its chief action on the auricles' Hence the predominant effect on the auricle seen after injection of quinamine

Quinamine does not increase the refractory period of the heart Chopra, Dikshit, and David (1928) found that while cinchonidine caused a marked prolonga tion of the refractory period, the action of cinchonine in this respect was inappre ciable Among the three dextro-rotatory alkaloids—cinchonine, quinidine and quinamine—only quinidine seems to cause a great increase in the refractory period of the heart

The irritability of the heart muscle is more or less decreased by all these three alkaloids

Summary

1 Quinamine causes a fall of blood-pressure by a direct depressant action on the heart muscle and a dilatation of the splanchnic blood vessels

2 An apparent stimulation of the auricle is caused by small doses, due to a *depression of the vagus centre* Larger doses cause a *depression of both* the auricle and ventricle by a direct action on the heart muscle

3 Quinamine has no influence on the latent period or the refractory period of the heart muscle

4 The irritability of the heart muscle is greatly depressed

5 No favourable effect is seen in cases of auricular fibrillations experi-mentally induced in anæsthetized animals

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THE CLINICAL VALUE OF THE ROSE BENGAL TEST FOR THE DETERMINATION OF THE TOTAL FUNCTIONAL CAPACITY OF THE LIVER

BY

M V RADHAKRISHNA RAO, MB, BS,

Research Fellow, Andhra University

(*From the Department of Pathology, Medical College, Vizagapatam*)

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WITH the rapid scientific advance of modern medicine, especially in the realms of physiology and biochemistry, new methods for the determination of the functional capacities of different organs have been introduced At present, these functional tests are amongst the newer and important acquisitions of the clinical laboratory Sir Humphry Rolleston (1931), in opening a discussion on the methods of determining liver-function and their value, at the Medical Society of London, observes —

'The increasing attention paid to the interpretation of symptoms and to functional disorders has taken investigation a stage further back than the detection of physical signs and structural changes, and is an attempt to study the earliest evidence of a departure from the normal and so to further the prevention of disease Functional tests have been extremely successful in organs, such as the stomach and kidneys, with comparatively simple functions, but their extension to the liver has brought about a very different position which is beset with difficulties'

The difficulties in the investigation of the liver-function are due to several factors The liver has a great reserve power with remarkable capacity to regenerate, particularly in chronic liver-cell damage An idea of the regenerative power of the liver can be obtained from the experiments of Mann and Bollman (1926), who found that, after removing 70 per cent of the normal liver of a dog, the remaining tissue increased and in a few weeks returned approximately to its pre-operative level Moreover, the liver has got varied functions, all of which may or may not be deranged in disease, this fact explains why similar pathologic conditions produce varied results when the same test is applied

While an extensive literature is available regarding liver-function tests, there does not appear to be any one reliable test to gauge exactly the functional capacity

of the organ in disease 'No test at present designed can be expected to give the knowledge which it would be most important of all to possess—the margin of safety between reasonably good functional activity and serious or even fatal breakdown' (Rolleston and McNee, 1929)

There is a great difference of opinion as to the value of many of the tests There does not appear to be any physiological basis for many of the tests employed to determine the functional capacity of the liver Many of the tests were devised to measure a supposed function of the organ, unless the function of the organ is definitely known, a functional test cannot be applied to determine such a function Excellent work on the physiology of the liver was done in recent years by Mann and his co-workers in the Mayo Clinic, but much of it is not yet useful to the clinician, to study the deviations from the normal in disease, at the bed-side

The trend of modern opinion, as revealed by the discussions of the Medical Society of London (1923 and 1931), only go to show that none of the tests so far devised should 'be regarded as of critical importance in the investigation of hepatic disease' In spite of these facts, the search for the useful functional tests of the liver still continues in all the clinical laboratories

The 'chromogogic function' of the liver, namely its capacity to eliminate certain dyes from the blood through the bile, when injected intravenously, has been used to determine the 'total or so-called global functional capacity' of the liver Abel and Rowntree (1909), while studying the pharmacological action of some phthaleins and their derivatives, found that phenol-phthalein was excreted in the urine and in the stools Further experimental studies with different variations in the phenol-phthalein molecule showed that the substance phenol-sulphonephthalein was mainly excreted by the kidneys, and the substance phenol-tetra-chlor-phthalein (first prepared by Orndoff and Black, 1909) was mainly excreted by the liver On the basis of these findings, Rowntree, Bloomfield, and Hurwitz (1913) formulated a test by using phenol-tetra-chlor-phthalein for estimating the hepatic efficiency On account of its technical difficulties this test was not adopted for routine use, until Rosenthal (1922 and 1924) introduced his important modification, in which the excretion of the dye was estimated by its rate of disappearance from the blood following intravenous injection, by comparison with a series of normal controls he was able to show that there was retention of the dye in diffuse damage of the parenchyma of the liver This work formed the starting point for estimating the hepatic efficiency by the 'chromogogic function' of the liver A further modification of the above test was introduced by Rosenthal and White (1925), who used bromsulphalein (phenol-tetra-bromphthalein sodium sulphonate) instead of phenol-tetra-chlor-phthalein This dye is extensively used in the estimation of hepatic function Though this 'chromogogic function' of the liver is not one of its normal functions, the introduction of this method as a functional test is of undoubted value in estimating hepatic efficiency Mann and Bollman (1926), commenting

on the liver-function tests, remark 'The physiologic basis for the use of the dyes which have been employed as tests of hepatic function is the fact that they appear to be excreted mainly by the liver Experimentally, the hepatic function could not be sufficiently diminished to show a definite relation of hepatic insufficiency to retention of dye Since, however, there is a definite retention of the dye in certain cases in man, this would appear to be one of those tests whose value can only be determined by its careful and controlled use clinically, as has been done by Rowntree and his associates'

Delprat (1918), working under Professor Herbert M Evans (Department of Anatomy, University of California), found that 'Rose Bengal' (di-iod-tetra-chlor-fluorescein), a dye belonging to the tri-phenyl-methane series, was entirely eliminated from the body by the cells of the hepatic parenchyma Further experimental work on the elimination of this dye from the blood as influenced by liver-injury was published by him in 1923 The clinical value of this test was investigated by Delprat, Epstein, and Kerr (1924) Both the experimental and clinical work showed that the rate of elimination of the dye following its injection into the circulation in normal subjects was fairly constant, while it was definitely delayed by any diffuse liver-injury This work formed the basis for the use of this dye as a liver-function test

Rose Bengal is a crystalloid substance, readily soluble in water or saline and entirely non-toxic in nature for the human tissues Professor Carl Schmidt showed that dyes of the fluorescein group, containing six or more halogens per molecule, were excreted entirely by the bile, while those containing less than six halogens per molecule may be excreted by the urine and bile Rose Bengal, containing as it does six halogens per molecule, is excreted by the bile only It has been shown by Norman and Schmidt (1920, 1922) that this dye has a 'photodynamic effect' on the red blood cells *in vitro* (i e, hæmolysing the red blood cells under the influence of direct sunlight), but Epstein, Delprat, and Kerr (1927) remark that 'this photodynamic effect does not seem to occur within the human body to an appreciable extent Furthermore, it has been our practice to keep tubes of blood, which contain some dye, in a darkened container until the cells have been separated from the dye-containing plasma by the centrifuge'

The original technique described by Delprat, Epstein, and Kerr (1924) was too tedious with its elaborate calculations, and a simplified modification of the test was adopted by Kerr, Delprat, Epstein, and Max Dunie Vitz (1925), for estimating the hepatic efficiency Epstein, Delprat, and Kerr (1927) further modified the technique, the details of which are described below

In taking this subject for investigation, my chief object is to study the value of this test (Rose Bengal) in the diagnosis of cirrhosis of the liver, and to determine its utility in the differentiation of ascites due to cirrhosis of the liver from other conditions resulting in ascites, e g, cardiac decompensation, kidney inefficiency,

chronic peritonitis, tubercular peritonitis, malignant peritonitis, etc A number of cases of cirrhosis of the liver, especially of the portal type, presented symptoms of secondary anæmia in their later stages, to determine whether anæmia *per se* has any effect on the rate of elimination of the dye, a number of control cases of secondary anæmia were included in this investigation Control cases of jaundice were similarly included to study the effect of jaundice on the excretion of the dye How far this test contributes to the study of hepatic cirrhosis, which I am investigating at present, is discussed in this paper

Procedure

(a) *Preparation of the solution* —It is always advantageous to prepare the solution of the dye and keep it ready for intravenous use after sterilization A one per cent solution of the dye in normal saline is prepared in a glass-stoppered measuring cylindrical jar, the solution is vigorously shaken so as to dissolve all particles of the dye and left overnight in the jar for the foam to settle The solution is then filtered and is filled in amounts of 15 c c into clean, dry test-tubes The mouths of the test-tubes are closed with plugs of cotton-wool and covered by wrapping a thin piece of paper, which is secured with thread These solutions in the test-tubes are then sterilized in the autoclave According to Delprat and Stowe (1931), these solutions can be used up to two months after preparation without any reactions developing in the patients, but it has been my practice not to use them after a month These solutions were prepared for me in the bacteriological department of the Medical College, with all aseptic precautions

(b) *Technique of the test* —(1) No special preparation of the patient is necessary for the injection of this dye solution The injection is usually given in the morning and the patient is merely prepared by being allowed no breakfast A cup of coffee is permitted early in the morning

(2) The dose of the solution to be injected depends upon the age of the person, children getting a smaller dose than adults In adults about 10 c c of the dye solution is injected, irrespective of the weight and age, into the median basilic vein

(3) The exact time of commencement of the injection of the dye into the vein is noted by starting a stop-watch and about 30 seconds are taken to inject the dye completely into the vein At the end of this time the syringe is quickly disconnected from the needle (which is still left in the vein) and another syringe, previously filled with about 10 c c of sterile (normal) saline, is connected to it The saline is then slowly injected, so that the syringe is empty at the end of two minutes Exactly at the end of the second minute, about 10 c c of blood are drawn into the syringe, which is then withdrawn with the needle from the vein Immediately the blood is transferred to a clean, dry, centrifuge tube containing a small quantity of finely powdered potassium oxalate The blood is gently mixed in the centrifuge

tube, which is fitted with a rubber-cork Vigorous shaking causes hæmolysis of the red blood cells and thus may vitiate the results

(4) The centrifuge tube containing the blood is quickly placed in a small tin padded with cotton-wool and covered with lid, so as to protect it from light This precaution is necessary on account of the photodynamic effect of this dye on the red blood cells already referred to

(5) Exactly at the end of the eighth minute (from the commencement of the injection) 10 c c of blood is drawn into another syringe from a large vein in the other ante-cubital fossa This is transferred to another centrifuge tube (containing potassium oxalate powder), which is similarly protected from light To avoid confusion, the tubes are numbered

(6) The clear plasma (which is coloured with the dye) is then separated from these two samples of blood by centrifuging them in an electric centrifuge, at a speed of about 2,000 revolutions per minute, for 15 to 20 minutes

(c) *Colorimetric analysis* —In the original test described by Epstein, Delprat, and Kerr (1927), the plasma, obtained after centrifuging the blood, was diluted with twice the volume of normal saline and the two samples were compared in the colorimeter But, any slight hæmolysis in either of the two samples resulted in imperfect matching of the colours, thus vitiating the results Hence the acetone precipitation method (described below) of Bloom and Rosenan (1924) was adopted by Delprat and Stowe (1931) —

From each of the samples 4 c c of clear plasma is pipetted into clean centrifuge tubes To each 8 c c of acetone is added, well mixed and left in the tin (which is covered with the lid) for 3 minutes They are then centrifuged To the clear supernatant fluid, a drop of 10 per cent sodium hydrate solution is added and well centrifuged after mixing The supernatant fluid is finally pipetted off into clean colorimetric cups According to Delprat and Stowe (1931), this alkalization sometimes helps in the full development of the colour of the dye in the plasma

The ' two minutes ' sample is taken as the standard and the ' eight minutes ' sample as the unknown and they are compared in the colorimeter The Klett's colorimeter has been used in this investigation

(d) *Calculation* —It has been shown by Epstein, Delprat, and Kerr (1927) that the maximum concentration of the dye was present in the blood at the end of two minutes after intravenous injection Hence the two minutes sample can be regarded as containing 100 per cent dye If the standard cup (containing the two minutes sample) be set at 20 in the colorimeter and if R is the colorimetric reading of the unknown (eight minutes sample), then, $\frac{20}{R} \times 100$ represents the percentage of dye present in the circulation at the end of eight minutes

It was shown by Delprat and his co-workers that in normal persons the eight minutes sample is usually 50 per cent (varying from 40 to 60 per cent) If the

dye is retained in higher concentrations in the circulation, it denotes hepatic derangement The ' total function ' of the liver is obtained from the formula, $200-(\frac{20}{R} \times 100) \times 2$, since ' twice the percentage of dye removed between the first and second samples equals the percentage of liver-function ' (Delprat and Stowe, 1931)

To avoid the possibilities of error due to hæmolysis in the colorimetric estimation of Rose Bengal in the blood, Snapper and Spoor (1927) suggested a spectroscopic method of evaluation of the dye But, by keeping the specimens of blood containing the dye in any light-free container, as described above, the results have been uniformly accurate and no hæmolysis occurred in any of my specimens

Results

To determine the clinical value of this test several patients were tested in the medical wards of the King George Hospital, Vizagapatam, and the results *in some of the typical cases* are given in the Table Only those cases in which the clinical diagnosis was very definite were selected for this study and these were further investigated thoroughly

In the few normal controls in which this test was applied, the excretion of the dye was within the normal limits given by Delprat and others

In cases of ascites due to cardiac decompensation of long duration the dye, present in the blood at the end of eight minutes, was at the highest limits of the normal or there was slight retention One patient (No 4), who came in with signs of acute heart failure, showed a reading within the normal limits, while another (No 7) with high blood-pressure and cardiac failure showed moderate retention of the dye The diagnosis was verified in one case (No 1) at autopsy

The excretion of the dye was normal in case No 8, in which the ascites was only a local manifestation of the general anasarca due to chronic nephritis

All the cases of ascites due to chronic peritonitis showed that the excretion of the dye was within the normal limits There was slight retention of the dye in one case (No 13) of tubercular peritonitis with ascites In one case (No 34) of malignant peritonitis with ascites, in which the diagnosis was made at operation (exploratory laparotomy) and later on verified at autopsy, the excretion of the dye was within normal limits

Jaundice, occurring as toxæmia of pregnancy, produced moderate retention of Rose Bengal and a similar result was observed in one case (No 15), in which the jaundice was the result of liver-damage, probably due to avitaminosis Moderate retention of the dye was definitely present in all cases of catarrhal jaundice

Malignant disease of liver produced marked retention of the dye, in one case (No 19), which showed discrete metastatic nodules in the liver, the elimination of the dye was within normal limits

TABLE

Showing the results of the Rose Bengal test in some typical cases

Serial number	Initials	Rose Bengal: Dye present at the end of 2nd min (Per cent)	Rose Bengal: Dye present at the end of 8th min (Per cent)	'Total function' of the liver (Per cent)	Disease	Case Notes (W R—Wassermann reaction V D B—Van den Bergh's reaction L T T—Lævulose tolerance test)
1	R G	100 0	60 0	80 0	Mitral stenosis with secondary heart failure—Ascites	H F, 30 years Breathlessness and swelling of the abdomen—1 year Marked fullness of the veins of the neck Systolic thrill at the apex of the heart, left border 5 inches from mid sternal line, rough creaky murmur accompanying the first sound, heard all over the præcordium, well marked systolic murmur conducted towards the axilla Liver enlarged to 4 fingers breadth below costal margin soft and tender Patient became worse and died in the hospital Macroscopic and microscopic appearances of the liver were those of typical chronic passive venous congestion
2	V A	100 0	66 5	67 0	Mitral regurgitation—Secondary heart failure—Ascites	H M, 36 years Breathlessness and swelling of the adbomen—5 years Marked cyanosis of the lips and finger tips, jugular pulsation present Diffuse wave over præcordium with a systolic thrill apex beat diffuse, diastolic thud at the apex first sound poor at the apex with a well marked, rough systolic murmur both sounds feeble at the base Congestion of both bases of the lungs Radiograph of the chest shows enlargement of left ventricle with fluid in right pleural cavity Electrocardiograph shows normal sinus rhythm and a right ventricular preponderance Liver enlarged to three fingers breadth below costal margin, tender and soft W R strong positive No diminution in the size of the liver after treatment with rest and digitalis, though the general condition improved
3	G A.	100 0	65 2	69 6	Double mitral—Secondary heart failure—Ascites	H F, 20 years Swelling of the legs and abdomen—1 year Marked cyanosis of the lips with œdema of the legs, pulsating veins at the root of the neck Marked systolic murmur in all the areas of the heart with gallop rhythm V D B indirect (faint), bilirubin content of the serum 0 6 units per c c A trace of albumin in the urine but no casts No diminution in the size of the liver after treatment

TABLE—*contd.*

Serial number	Initials	Rose Bengal: Dye present at the end of 2nd min (Per cent)	Rose Bengal: Dye present at the end of 8th min (Per cent)	'Total function' of the liver (Per cent)	Disease	Case Notes (W R—Wassermann reaction V D B—Van den Bergh's reaction L T T—Lævulose tolerance test)
4	B S	100 0	46 3	106 4	Congestive heart failure—Secondary to syphilitic myocarditis—Ascites	H F, 30 years Visible pulsation and marked fullness of the vessels at the root of the neck Left border of the heart 7 inches from mid sternal line in 6th interspace, well marked systolic murmur at the apex, conducted towards the axilla, reduplication of second pulmonary sound at the base Liver enlarged to 3 fingers breadth below costal margin, tender and soft, abdomen distended with free fluid Trace of albumin in the urine W R strong positive
5	P A	100 0	60 9	78 2	Myocarditis (Syphilitic ?)—Ascites	H F, 20 years Fullness of the veins of the neck, swelling of the feet and abdomen Left border of the heart 6 inches from mid sternal line, well marked systolic murmur at the apex conducted towards the axilla, systolic murmur in pulmonary area Liver enlarged to 3 fingers breadth below costal margin and tender W R positive
6	Ab	100 0	65 8	68 4	Myocarditis (cardio vascular syphilis)—Ascites	Ch M, 30 years Markedly cyanosed, fullness of the veins of the neck, swelling of the legs and abdomen Left border of the heart in the anterior axillary line, heart sounds rapid, first sound short and sharp in mitral and tricuspid areas, weak in pulmonary and aortic areas Congestion of both the bases of the lungs Radiograph of the chest shows a dilated heart Electro cardiograph—normal sinus rhythm, interpolated auricular premature contractions in Lead I, T wave inverted in Leads I and II and absent in Lead III, amplitude in all the waves very poor especially in Lead III Liver enlarged to 2 fingers-breadth below costal margin and tender W R strong positive V D B direct delayed (faint), bilirubin content of the serum 0 6 units per c c No albumin in urine Patient showed no improvement and died Autopsy not permitted

7	D S	100 0	82 0	36 0	Congestive heart failure—Secondary to arteriosclerosis and high blood pressure—Ascites	H M, 10 years Fullness of the veins of the neck Swelling of the feet and abdomen Left border of the heart 1" outside nipple line first sound weak in all the areas, second sound accentuated and reduplicated at the base, systolic murmur in mitral and tricuspid areas Blood pressure 170—115 mm W R strong positive V D B indirect (faint), bilirubin content of the serum 0 6 units per c c Radiograph of the chest shows a dilated heart with atheromatous changes in the aorta throughout its course in the thorax No albumin in the urine Kidney function fair Patient improved after administration of digitalis and salvrgan
8	C A	100 0	41 6	116 6	Chronic nephritis and arteriosclerosis—Ascites	H M, 50 years Swelling of the abdomen and breathlessness—1 years Puffiness of the face, anæmia and œdema of the extremities Liver and spleen not palpable even after paracentesis of the abdomen Systolic murmur in mitral area of the heart second pulmonary sound accentuated number of extra systoles heard at irregular intervals Blood pressure 200—140 mm Pulse tension high, radial artery thickened Blood urea 60 0 mg , non protein nitrogen, 46 14 mg , creatinin, 2 4 mg per cent Fundi of the eyes show retinitis along the superficial temporal vessels Urine—pale, watery, sp gr 1012 with plenty of albumin microscopically number of R B C leucocytes, fatty hyaline and granular casts Urea concentration test shows diminished renal function Both bases of the lungs congested
9	Y K	100 0	59 5	81 0	Chronic peritonitis—Ascites	H M, 35 years Markedly emaciated and anæmic No prominent veins over the anterior abdominal wall ascites present liver and spleen not palpable even after paracentesis of the abdomen Heart sounds soft hæmic murmurs in pulmonary and mitral areas Congestion of both the bases of the lungs W R weak positive V D B indirect (faint) Hæmoglobin 30 per cent Liver function not impaired (Lævulose tolerance test and Widal's hæmo clasic crisis) Kidney function good, no albumin urobilin or urobilinogen in urine Abdomen rapidly filling up paracentesis was done 9 times in hospital No improvement in patient's condition
10	M R	100 0	56 55	90 0	Peritonitis, secondary to pelvic cellulitis—Ascites	H F, 30 years Swelling of the abdomen (11 days duration) nine days after a miscarriage in the fifth month Abdomen tender distended with free fluid, flanks bulging and umbilicus flattened out, spleen and liver not palpable Temperature 99 5°1 Vaginal examination—cervix pushed against symphysis by a boggy mass (old blood clots ?) in the Douglas pouch Examination of ascitic fluid shows it to be inflammatory in origin W R of blood and ascitic fluid strong positive V D B negative Diminished resonance with feeble breath sounds over both the bases of the lungs

TABLE—*contd*

Serial number	Initials	Rose Bengal: Dye present at the end of 2nd min (Per cent)	Rose Bengal: Dye present at the end of 8th min (Per cent)	'Total function' of the liver (Per cent)	Disease	Case Notes (W R —Wassermann reaction V D B —Van den Bergh's reaction L T T —Lævulose tolerance test)
11	Ac	100 0	45 0	110 0	Chronic pleurisy with effusion and chronic peritonitis (Polyserositis ?)	H F, 25 years Cyanosis of the lips, puffiness of the face and œdema of the legs Effusion into the right pleural cavity up to the apex, confirmed by skiagram and exploratory puncture Heart normal No visible veins over anterior abdominal wall, free fluid present in the abdomen, liver pushed down by pleural effusion, spleen not palpable W R negative No jaundice Urine normal General condition improved after administration of digitalis and salyrgan, ascites and pleural effusion still present
12	C V	100 0	57 5	85 0	Chronic peritonitis and chronic pleurisy with effusion (Polyserositis ?)	H M, 30 years Puffiness of the face, œdema of both the legs and slight anæmia, no jaundice Abdomen distended with free fluid, liver and spleen not palpable even after tapping the abdomen, abdomen rapidly filling up requiring frequent tappings Heart-sounds weak Pleural effusion on the right side confirmed by skiagram and exploratory puncture W R negative V D B negative Kidney function good, a trace of albumin in urine, but no urobilin Biochemical examination of the blood, normal, plasma proteins low No improvement in patient's condition
13	K S	100 0	66 75	66 5	Tubercular peritonitis—Ascites	H M, 20 years Swelling of the abdomen—4 months Bilateral tuberculosis of the lungs, spontaneous pneumothorax of the left side with active signs of tuberculosis in the right lung, confirmed by radiographic examination Wasting of the left side of the chest with scoliosis in upper thoracic region, with the convexity to the right No veins on the anterior abdominal wall, free fluid in the abdomen, liver and spleen not palpable Thickened and rolled omentum felt as a sausage shaped mass Ascitic fluid sp gr 1022, albumin, 4 per cent, inoculated guinea pig under observation Patient improved with ultra violet rays exposure to the abdomen after paracentesis and injection of oxygen into the abdominal cavity

14	K S	100 0	78 6	42 8	Jaundice—Toxæmia of pregnancy	H F, 16 years Primipara, 7th month of pregnancy Jaundice—1 month Liver just palpable and tender Motions, dirty clay-coloured Heart and lungs normal Bile salts, bile pigments and urobilin in urine V D B direct immediate, bilirubin content of the serum 5 05 units W R negative Urea concentration test and urine diastose content normal Plasma cholesterol increased (250 mg per cent) Fragility of red cells diminished Blood counts normal Blood coagulation time delayed No improvement after a stay of three weeks in hospital
15	K R	100 0	86 2	27 6	Jaundice—Avitaminosis	H M, 22 years Night blindness—2 years Staphyloma right eye and leucoma adherans left eye, no vision in left eye, hand movements only perceptible in right Xerosis of the conjunctiva Liver just palpable, no ascites General condition very poor First sound of the heart soft in all the areas, systolic murmur at the apex Congestion of both bases of the lungs W R negative V D B direct immediate, bilirubin content of the serum 9 375 units Fragility of red cells normal Blood counts show secondary anæmia, plasma cholesterol 212 8 mg per cent Liver function impaired (L T T rise of blood sugar over fasting level, 45 7 mg per cent) Dark ground illumination and culture of blood negative for spirochæta icterohæmorrhagica Kidney function normal bile pigments present but no albumin in urine culture of urine negative for spirochæta icterohæmorrhagica No improvement after a stay of one month in hospital
16	Ra	100 0	72 5	55 0	Catarrhal jaundice	H M, 28 years Jaundice—15 days Liver not palpable Motions pale whitish Bile salts bile pigments and urobilin in urine W R *strong positive* V D B *direct immediate* Liver function slightly impaired (L T T rise of blood sugar over fasting level, 26 0 mg per cent) Jaundice cleared after the administration of hexamine and concentrated mag sulph solutions as recommended by Hurst
17	Ka	100 0	79 7	40 6	Catarrhal jaundice	H M, 25 years Jaundice of six days duration, after fever lasting for two days Icterus of all visible mucous membranes Liver not palpable W R strong positive V D B direct immediate Liver function slightly impaired (L T T rise of blood sugar over fasting level, 26 5 mg per cent) Motions pale whitish Bile salts, bile pigments and urobilin in urine Jaundice cleared after treatment

TABLE—*contd*

Serial number	Initials	Rose Bengal: Dye present at the end of 2nd min (Per cent)	Rose Bengal: Dye present at the end of 8th min (Per cent)	'Total function' of the liver (Per cent)	Disease	Case Notes (W R—Wassermann reaction V D B—Van den Bergh's reaction L T T—Lævulose tolerance test)
18	V R	100 0	95 5	9 0	Primary malignant disease of the liver	H M, 40 years Swelling in the epigastric region—2½ months Marked cachexia and deep icterus of all visible mucous membranes Liver enlarged three inches below costal margin, very hard and tender, few rounded nodules varying in size were palpable through the thin abdominal wall W R negative V D B direct positive Blood counts show secondary anæmia Heart-sounds soft with faint systolic murmurs in mitral and pulmonary areas Injections of emetine empyrically given (though there was no history of dysentery) with no benefit Patient developed petechial hæmorrhages over the fore head and temporal regions No improvement in patient's condition (Further history of this patient could not be traced after his discharge from the hospital)
19	B N	100 0	57 1	85 8	Metastatic malignancy of the liver (primary in right testicle)	H M, 40 years Swelling of the abdomen—6 months General condition very poor with icterus of all mucous membranes Right testicle had not descended into the scrotum, a hard swelling present above the pubis, just to the right of the middle line (in the region where the retained testicle was situated) Liver enlarged to four fingers breadth below costal margin, surface irregular with rounded masses, which could be palpated Prominent veins in the epigastric region and over right side of the abdomen extending to the side of the thorax W R positive V D B direct immediate Bile salts, bile pigment and urobilin in urine No improvement in patient's condition even after deep *X*-ray therapy

20	Y E	100 0	30 8	138 4	Myocarditis and anæmia	H F, 35 years, Swelling of the abdomen and legs—1 month Markedly anæmic No jaundice Liver and spleen not palpable, ascites present Dilated heart with hæmic murmurs in all the areas Signs of active tuberculosis in the upper lobes of both the lungs, confirmed by skiagram V D B negative Blood counts show secondary anæmia, *hæmoglobin 35 per cent* Ankylostome ova in stools Kidney function good, urine normal Liver function good as shown by L T T
21	B R	100 0	44 0	112 0	Ankylostomiasis—Secondary anæmia	H M, 40 years Breathlessness and œdema of the legs—1 year Markedly anæmic Liver and spleen not palpable, no ascites Dilated heart with hæmic murmurs V D B direct delayed (faint) Hæmoglobin, 35 per cent No albumin in urine Ankylostome ova in stools Examination of blood shows secondary anæmia
22	N T	100 0	36 3	127 4	Ankylostomiasis—Secondary anæmia	H M, 35 years *Pallor* of visible mucous membranes—10 months Liver not palpable Heart sounds weal V D B indirect (faint) W R negative Blood counts show secondary anæmia Ankylostome ova in stools, no albumin in urine
23	Ve R	100 0	54 0	92 0	Ankylostomiasis—Secondary anæmia	H M, 35 years Pallor of visible mucous membranes—10 months Marked secondary anæmia V D B negative Ankylostome ova in stools
24	K P	100 0	62 0	76 0	Ankylostomiasis—Secondary anæmia	H M, 40 years Breathlessness—1 year Marked secondary anæmia Hæmoglobin 30 per cent Liver not palpable Stools contain ankylostome, roundworm and whipworm ova
25	B G	100 0	63 0	74 0	Ankylostomiasis—Secondary anæmia	H M, 35 years Breathlessness—3 years Marked secondary anæmia Hæmoglobin, 25 per cent Liver and spleen not palpable Ankylostome ova in stools

TABLE—*contd*

Serial number	Initials	Rose Bengal: Dye present at the end of 2nd min (Per cent)	Rose Bengal: Dye present at the end of 8th min (Per cent)	'Total function' of the liver (Per cent)	Disease	Case Notes (W R—Wassermann reaction V D B—Van den Bergh's reaction L T T—Lævulose tolerance test)
26	M A	100 0	66 7	66 6	Ankylostomiasis—Secondary anæmia	H F, 20 years Pallor of visible mucous membranes—4 years Liver not palpable, spleen enlarged to the level of the umbilicus Marked anæmia Hæmoglobin, 20 per cent W R strong positive V D B direct delayed (faint) Trace of albumin in urine Ankylostome ova in stools Liver-function good (L T T rise of blood-sugar over fasting level, 10 3 mg per cent)
27	K K	100 0	92 6	14 8	Ankylostomiasis—Ascites	H M, 40 years Swelling of the abdomen—3 months Extremely anæmic with slight icterus of the conjunctiva Free fluid in the abdomen, liver not palpable Spleen enlarged to four fingers-breadth below costal margin, no veins seen on the anterior abdominal wall Heart sounds feeble in all the areas W R negative V D B direct delayed Blood count show secondary anæmia Hæmoglobin 30 per cent, blood coagulation time delayed Kidney function good, urine contains urobilin, but no albumin Number of ankylostome ova in stools Liver function slightly impaired (L T T rise of blood sugar above fasting level, 23 4 mg per cent)
		100 0	88 6	22 8	(Fatty degeneration of liver ?)	
28	M B	100 0	95 0	10 0	Hypertrophic biliary cirrhosis—Ascites	H M, 30 years Jaundice—1½ years No history of biliary colic at any time Abdomen distended with free fluid (duration—3 months) Liver palpable to 5 fingers breadth below xiphisternum, hard and finely granular on the surface, spleen palpable to two fingers breadth below costal margin ascitic fluid deeply bile stained Heart and lungs normal W R negative V D B direct immediate, bilirubin content of the serum 6 87 units per c c Bile salts, bile pigments and urobilin in urine Motions brownish white and offensive Liver function impaired (L T T rise of blood-sugar above fasting level, 37 7 mg per cent) No improvement in patient's condition

29	R A	100 0	98 6	2 8	Portal cirrhosis—Ascites	H M, 30 years Swelling of the abdomen—6 months Poorly nourished, with a haggard look, no pallor of mucous membranes, subicteric tint of conjunctiva Abdomen distended with free fluid in it, only the left lobe of the liver was palpable to 3 fingers breadth below xiphisternum, hard and granular on its surface, spleen markedly enlarged to 2 inches below the level of the umbilicus Heart sounds weak Congestion of both the bases of the lungs W R positive V D B direct delayed, bilirubin content of the serum 3 3 units per c c Urobilinogen present in urine, but no albumin Liver function impaired (L T T rise of blood sugar over fasting level, 43 6 mg per cent) Patient's condition became worse and died in hospital Autopsy showed portal cirrhosis of the liver (verified by microscopic examination)
30	S K	100 0	92 6	14 8	Portal cirrhosis—Ascites	H M, 35 years Swelling of the abdomen—2½ months Moderately nourished, not anæmic, no jaundice Dilated and prominent veins over the anterior abdominal wall, ascites present, liver not palpable spleen enlarged to two fingers breadth below costal margin Cardiac and respiratory embarrassment due to ascites, otherwise normal W R strong positive V D B direct delayed Blood coagulation time delayed Urobilin and urobilinogen in urine, no albumin Liver function impaired (L T T rise of blood sugar over fasting level, 38 2 mg per cent) Abdomen rapidly filling up necessitating frequent tappings No improvement in patient's condition
31	N K	100 0	98 4	3 2	Cirrhosis liver—Ascites	H M, 45 years Hepatic facies present, no jaundice Markedly dilated veins over the anterior abdominal wall extending to the sides of the chest liver and spleen not palpable, abdomen distended with free fluid in the peritoneal cavity Heart sounds feeble W R strong positive V D B direct delayed Liver function impaired (L T T rise of blood sugar above fasting level 31 8 mg per cent) Urine contains indican and urobilin no albumin Examination of ascitic fluid shows it to be a transudate No improvement in patient's condition
32	S J	100 0	76 5	47 0	Cirrhosis liver—Ascites	H M, 35 years Swelling of the abdomen—1½ months Moderately nourished, not anæmic, with jaundice of the conjunctiva Veins of the anterior abdominal wall, dilated and prominent, ascites present liver not palpable, spleen enlarged to a finger breadth below the costal margin Heart-sounds good Congestion of both the bases of the lungs W R positive V D B biphasic, bilirubin content of the serum 6 755 units per c c Liver function impaired (L T T rise of blood sugar over fasting level, 50 2 mg per cent) Urobilin and urobilinogen in urine, no albumin Patient's condition became worse after paracentesis of the abdomen

TABLE—*concld*

Serial number	Initials	Rose Bengal: Dye present at the end of 2nd min (Per cent)	Rose Bengal: Dye present at the end of 8th min (Per cent)	'Total function' of the liver (Per cent)	Disease	Case Notes (W R —Wassermann reaction V D B —Van den Bergh's reaction L T T —Levulose tolerance test)
33	W V	100 0	98 5	3 0	Syphilitic cirrhosis liver (no ascites)	H M, 11 years Slightly emaciated, visible mucous membranes jaundiced, slightly anæmic Abdomen prominent, no ascites, liver enlarged to 4 fingers breadth below costal margin hard, with a smooth surface and a rounded edge Spleen enlarged to 4 fingers breadth below costal margin and hard Nothing abnormal in heart and lungs W R strong positive V D B direct immediate, bilirubin content of the serum, 7 935 units per c c Fragility of red blood cells diminished Blood shows secondary anæmia Liver function impaired (L T T rise of blood sugar over fasting level 27 2 mg per cent) Urine contains urobilin urobilinogen and a trace of albumin This patient had fever in the hospital and examination of the blood showed M T parasites
34	Ra	100 0	59 5	81 0	Malignant peritonitis	H M, 38 years Swelling of the abdomen—20 days abdomen markedly distended with free fluid, a solid tumour could be felt in the epigastric region, spleen and liver not palpable prominent veins present over the abdominal wall, blood stained fluid drawn by exploratory puncture W R negative V D B direct delayed A trace of albumin in the urine but no bile pigments, bile salts or urobilin Cardiac and respiratory embarrassment due to fluid in the abdomen otherwise nothing abnormal Exploratory laparotomy showed number of small deposits in the parietal peritoneum with blood stained fluid in the abdomen microscopic examination of a piece of parietal peritoneum (Biopsy) showed malignant carcinomatous deposits with chronic inflammation Autopsy showed a huge malignant growth (primary) in the mesentery

The elimination of the dye was normal in cases of secondary anæmia due to ankylostomiasis, slight retention of the dye was present in cases (Nos 24, 25 and 26), in which the anæmia persisted for a long time One case (No 27) with extreme anæmia due to ankylostomiasis and signs of hepatic insufficiency, showed marked retention of the dye, the test was repeated with the same result

All the cases of cirrhosis of the liver showed retention of the dye moderate retention was present in case No 32, while marked retention was present in cases Nos 28, 29, 30, 31 and 33 The diagnosis of portal cirrhosis was verified in one case (No 29) at autopsy

Comment

(A) *On the results*

(*a*) The results obtained in portal cirrhosis of the liver, where there is marked retention of the dye without bilirubinæmia, are in agreement with those of Epstein, Delprat, and Keri (1927) Similar observations were made by Greene, McVicar, Snell, and Rountree (1927), with phenol-tetra-chlor-phthalein and by Foley (1930) with brom-sulphalein Working with brom-sulphalein, Greene, Snell, and Walters (1931) remark that the results of brom-sulphalein test in portal cirrhosis are so constant, that one should be cautious in attributing ascites to a hepatic lesion, in the absence of such a positive test' The retention of the dye is constant and the dissociation of function (i e , retention of the dye without an increase in the bilirubin content of the blood) characteristic, in cases of portal cirrhosis of the liver with ascites

In seeking for an explanation for this marked retention of the dye in portal cirrhosis, the following factors have to be taken into consideration (1) diminished flow of blood through the cirrhotic liver, (2) structural alterations in the hepatic lobule, and (3) degeneration of the liver-cells

(1) *Diminished flow of blood through the cirrhotic liver* —McIndoe (1927), studying the vascular lesions of portal cirrhosis by the corrosion method described by Counseller and McIndoe in 1926 (where casts of the liver were produced by injecting its channels with celloidin solutions and subsequently destroying the parenchyma by corrosives), showed that one of the most obvious changes in portal cirrhosis is the marked diminution in the total hepatic vascular bed By perfusing the portal vein in cirrhotic livers with physiologic sodium chloride solution at the probable pressure in the portal vein in cirrhosis (between 10 and 20 mm of mercury), McIndoe (1927) showed that ' thirteen per cent is the most that is recovered from the hepatic vein, in some cases none, in contrast to 100 per cent in the normal liver' As the intra-hepatic collaterals are given off the portal vein before the sinusoidal bed is reached, it is quite certain that none of the fluid recovered from these channels comes in contact with the hepatic parenchyma since it is shunted

as through a by-pass before it reaches its normal destination Some of the fluid recovered from the hepatic veins may or may not have passed through the nodules of hepatic cells, but even if all of it did, the amount would be extremely small' Thus, the portal blood-supply of the hepatic parenchyma is reduced to a minimum and the hepatic artery is the main source of supplying sufficient blood to carry on the normal metabolism The great bulk of the portal blood reaches the heart through the collateral channels, resulting in delayed presentation of the dye to the hepatic parenchyma This factor explains to some extent the marked retention of the dye in cases of portal cirrhosis

(2) *Structural alterations in the hepatic lobule* —The regenerative features which are commonly seen in portal cirrhosis were demonstrated by Kretz (1905) and MacCallum (1904) As a result of the degenerative and regenerative changes taking place in the liver, together with an increase of fibrous tissue, the normal structure of the hepatic lobule is completely lost ' Instead of lying in a continuous sheet between the portal and hepatic venous trees, and in the direct pathway of the afferent and efferent blood-vessels, the hepatic cell mantle is now broken up into innumerable irregular independent and anastomosing nodules, virtually all new formations, consisting of twisting columns of cells with narrow tortuous sinusoids, without a suggestion of the usual radiate arrangement' (McIndoe, 1927) By injecting coloured gelatin into the portal vein McIndoe (1927) showed that the new hyperplastic nodules remain uncoloured, showing thereby that they are ' almost completely side-tracked from the portal blood-supply ', when injected through the hepatic artery, the coloured gelatin reaches the hyperplastic nodules with difficulty Thus, the hepatic artery forms the only source of blood-supply to the remaining liver-tissue Hence, when Rose Bengal is injected intravenously (in cases of portal cirrhosis), it is conveyed to the liver-cells mostly by the hepatic artery, and as the amount of blood circulating in the hepatic artery is much less compared to that of the portal vein [25 to 30 per cent of the inflow of blood to the liver is contributed by the hepatic artery in the dog—(Bauer *et al*, 1932)], only a limited amount of the dye is presented to the liver-cells in a particular time As already shown the amount of portal blood circulating in the liver in cases of portal cirrhosis is greatly diminished and even the dye (Rose Bengal) circulating in this diminished quantity of the blood is not presented to the newly formed liver-cells, as they are side-tracked from the portal blood-supply Thus, the alterations in the structure of the liver as a result of the different changes taking place in it in portal cirrhosis, result in a delay in the excretion of the dye

Another structural alteration found in the regenerated hepatic tissue has recently been reported by Althausen (1931), who pointed out that in certain types of cirrhosis (such as toxic cirrhosis) of the liver, the hyperplastic nodules lack connection with the bile channels This results in a marked retention of the

Rose Bengal dye, though the liver-cells in the regenerated tissue are able to utilize the carbohydrates in the normal manner

(3) *Degeneration of the liver-cells* —A constant feature, seen microscopically in most of the sections of the liver in portal cirrhosis, is the fatty change in the hyperplastic nodules The injurious agent which caused the cirrhosis is always at work, unless it can be stopped, and hence the liver-cells are always 'susceptible to injury, and for that reason they are often found at the death of the individual loaded with fat or injured in some other way Some of these injuries may be of the series which is still at work, adding to the changes which bring about the cirrhosis, but others may be independent' (MacCallum, 1928) Hence, the chromogogic function of the liver is greatly diminished on account of the reduced amount of healthy liver-tissue present in portal cirrhosis

Thus, the degree of retention of the dye in portal cirrhosis is directly proportional to the pathological processes taking place in the liver It denotes to a certain extent the balance between the degenerative and regenerative processes in the liver and constitutes a very good laboratory evidence of hepatic insufficiency, especially in the absence of jaundice The retention of the dye does not seem to correspond to the degree of ascites, which denotes only the circulatory disturbance in portal cirrhosis The secondary anæmia, which is seen in most of the cases of portal cirrhosis in its later stages, cannot by itself account for the marked retention of the dye found in these cases

While retention of the dye constitutes a very good evidence in the diagnosis of portal cirrhosis, it is not of such great significance in the prognosis of the disease Mann and his associates have shown, from their experimental researches, that only a small amount of the liver-tissue (much less than is present in advanced cases of portal cirrhosis), properly nourished, is sufficient to carry on the ordinary metabolism of the body Moreover, 'majority of patients with portal cirrhosis do not die from hepatic insufficiency but from complicating disorders, such as circulatory failure, hæmorrhage, ascites, and intercurrent diseases' (Rowntree, 1927)

(*b*) The marked retention of the dye, in cases of hypertrophic biliary cirrhosis and syphilitic cirrhosis of the liver, can be explained by the diffuse involvement of the parenchyma in these diseases Working with phenol-tetra-chlor-phthalein, Greene, McVicar, Snell, and Rowntree (1927) showed that retention of the dye occurred uniformly in all cases of biliary cirrhosis O'Leary, Greene, and Rowntree (1929) pointed out that practically all cases of syphilitic cirrhosis of the liver showed retention of the dye (phenol-tetra-chlor-phthalein or brom-sulphalein)

(*c*) A slight retention of the dye was present in most of the cases of long-standing cardiac failure with passive congestion of the liver Foley (1930) obtained similar results with brom-sulphalein, in cases of passive congestion of the liver resulting from cardiac decompensation But, Epstein, Delprat, and Kerr (1927)

remark that 'chronic passive congestion of the liver does not seem to prevent the absorption of Rose Bengal and the curves are within normal limits' There is certain amount of fibrosis (the so-called cyanotic induration, or the cardiac type of cirrhosis) in cases of chronic venous congestion of the liver, apart from this, necrosis of the cells in the inner zone (surrounding the intra-lobular vein) of the hepatic lobule is seen in the later stages of the condition These pathological changes in the liver explain the slight retention of the dye present in these cases

(*d*) The Rose Bengal test forms a very useful aid in the investigation into the causation of ascites The excretion of the dye is within normal limits or there is only slight retention, in cases of ascites due to cardiac decompensation, nephritis or peritonitis—simple, tubercular or malignant, while there is marked retention in cases of ascites resulting from cirrhosis of the liver Thus, this test is of value in differentiating cases of ascites due to cirrhosis of the liver from other conditions resulting in ascites

(*e*) Jaundice, which is the visible manifestation of hepatic derangement in cases of toxæmias of pregnancy, avitaminosis and catarrhal jaundice, is always accompanied by moderate retention of the dye This can be explained by the fact that the parenchyma of the liver is definitely damaged in all these conditions

(*f*) The marked retention of the dye in cases of malignant disease of the liver is due to the diffuse destruction of the hepatic parenchyma The rate of elimination of the dye in cases of secondary malignant deposits in the liver depends upon the amount of functionally active liver-tissue still remaining 'Discrete scattered metastatic nodules in the liver do not interfere with its ability to eliminate the dye, but diffuse replacement of the liver-tissue by carcinoma very definitely causes a retention of the dye in the blood-stream' (Epstein, Delprat, and Kerr, 1927)

(*g*) The retention of the dye, in cases of long-standing anæmia due to ankylostomiasis, is probably due to the fatty degeneration of the liver, which is seen in the later stages of the disease

(B) *On the technique, etc*

Much clearer solutions are obtained for colorimetric analysis by the acetone precipitation method described by Delprat and Stowe (1931), yet, in a few cases the extraction of the colour of the dye by the acetone seemed to be imperfect and the results obtained were lower than those obtained by diluting the coloured plasma with normal saline I have used both the methods and found the former to be undoubtedly better in most of the cases

In cases of intense jaundice there is some difficulty in matching the colours, as the colour of the dye in the plasma is superimposed on the colour of the bile pigments present in it

No useful purpose is served in calculating the 'total function' of the liver from the percentage of dye present in the blood at the end of the eighth minute by the formula given by Delprat and Stowe (1931) A marked retention of the dye does not necessarily mean that all the other functions of the liver are damaged to an equal extent As pointed out by Althausen (1931), in toxic cirrhosis of the liver the carbohydrate metabolism is normal, though there is marked retention of Rose Bengal Taking, for instance, case No 31, the 'total function' of the liver comes to 3 2 per cent (according to the formula of Delprat and Stowe), but this patient did not exhibit any symptoms of hepatic insufficiency As already pointed out retention of the dye is of great significance in the diagnosis of cirrhosis of the liver, but its value in the prognosis is very limited It would perhaps be better if the retention of the dye is simply noted down as slight, moderate or marked, in each case, a classification of this type denotes only the chromogogic function' of the liver and does not encroach on the other functions of the liver

The dye is entirely non-toxic to the human tissues and no untoward symptoms were seen in any of the cases after the intravenous injection according to the dosage already given One patient (No 15) had a severe rigor after the injection, while another patient (No 26) showed a rise of temperature to 102 5 F , none of the others showed any reaction after the intravenous injection of the dye

(C) *On the value of the test*

Retention of the dye is present in varied conditions, and the test does not aid in differentiating the several conditions producing this retention It is a valuable aid in the study of hepatic diseases when taken in conjunction with the clinical observations and the results of other tests 'It is analogous to the phenol-sulphoneph-thalein test of kidney-function, in being not a test of any specific type of organ damage, but a measure of the totally remaining functionally active tissue' (Delprat and Stowe, 1931) The value of this test is briefly summarized by Barker (1930) —

'One of the dyes used by Evans, namely, "Rose Bengal", would seem to fulfil, better than any other yet used, the ideal requirements of a dye for the study of liver function (as formulated by Delprat), since it is a non toxic, crystalloid substance, which is eliminated from the body by the cells of the liver parenchyma and yet remains in the circulating blood long enough after injection to permit of determinations of its concentration in the blood plasma the recent simplification of the method makes the test easily applicable by any one accustomed to the methods of the modern clinical laboratory'

Summary and conclusions

(1) Rose Bengal (di-iod-tetra-chlor fluorescein), a dye belonging to the triphenylmethane series, has been utilized to study the chromogogic function of the liver for determining the total functional capacity of the organ

(2) The modified technique described by Delprat and Stowe (1931) was adopted in this investigation and the test is described in detail

(3) The dye is entirely non-toxic to the human tissues and no untoward symptoms were seen in any of the cases after intravenous injection of the dye in doses of 100 mg for the adult

(4) Marked retention of the dye was found in cases of cirrhosis and malignant disease of the liver In cases of metastatic malignancy of the liver the excretion of the dye depends upon the amount of functionally active liver-tissue still remaining Marked retention of the dye without bilirubinæmia constitutes an important observation in cases of decompensated portal cirrhosis of the liver The causes of this marked retention of the dye in portal cirrhosis are discussed and it is shown that the secondary anæmia, which is seen in the later stages of the disease, cannot account for this marked retention

(5) Jaundice associated with toxæmias of the liver always produced retention of the dye

(6) Slight retention of the dye was seen in long-standing cases of passive congestion of the liver resulting from cardiac decompensation and in cases of ankylostomiasis, in which the secondary anæmia had persisted for a long time

(7) The test is a valuable aid in differentiating ascites due to cirrhosis of the liver from other conditions producing ascites, e g , cardiac decompensation, nephritis, or peritonitis—simple, tubercular or malignant

(8) The results obtained in this investigation are in agreement with those obtained by the originators of this test The test is more likely to be positive in those conditions in which there is diffuse destruction of the hepatic parenchyma, than in those with localized affection The test does not show any specific type of organ damage, but only denotes the amount of functionally active liver-tissue still present

(9) It is pointed that, while the retention of the dye is of great significance in the diagnosis of cirrhosis of the liver, its value in the prognosis is very limited It is suggested that no useful purpose is served in calculating the ' total function ' of the liver, from the percentage of dye present in the blood at the end of eight minutes, by the formula given by Delprat and Stowe (1931)

(10) Taken in conjunction with the clinical observations and the results of other tests, the Rose Bengal test constitutes a valuable aid in the study of hepatic diseases

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[Since sending this paper to the press, four cases (2 cases of portal cirrhosis with ascites, one case of syphilitic cirrhosis with jaundice, and one case of toxic jaundice due to intravenous injections of 'Neo Salvarsan') were observed, in which the urine was slightly coloured with the dye after the intravenous injection of Rose Bengal. All these cases showed moderate retention of the dye at the end of the eighth minute.—M. V. R.]

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THE METABOLISM OF CAROTENE IN DIFFERENT ANIMALS

BY

BASHIR AHMAD, M SC, PH D, A I C,

AND

KHAZAN SINGH MALIK, M SC

(From the University Chemical Laboratories, Lahore)

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INTRODUCTION

It is now well known that carotene can replace vitamin A in the diet of the rat (Euler, Euler, and Hellstrom, 1928, Moore, 1929, Collison, Hume, McLean, and Smith, 1929, Drummond, Ahmad, and Morton, 1930) On feeding rats with large doses of carotene the pigment is metabolized in the organism giving rise to the familiar form of vitamin A, which is stored in the liver when in excess of the requirements of the animal Further work along these lines has now proved that carotene is the precursor of vitamin A and the vitamin arises in the animal body from the carotene of plants (Ahmad, 1930)

In a previous study of the metabolism of carotene (Ahmad and Drummond, 1931) it was found that certain factors profoundly affect the mechanism of the absorption of carotene and its conversion into vitamin A It also appeared that certain animals are unable to metabolize carotene for their vitamin A requirements (Ahmad, 1931) This observation raised the important question whether all animals are able to obtain their requirements of vitamin A from the plant pigment carotene, or whether some of them, that have lived through the ages upon animal foods, have lost this function during the course of evolution How far does this capacity of assimilating carotene for vitamin synthesis vary in different animal species and what is the position of man with regard to such synthesis? To solve

this problem a comparative study of the physiological power of certain animals to convert carotene into vitamin A has been undertaken

Experimental

A quantity of carotene was prepared from carrots The roots were cut into thin flakes and dried in the shade under a hot blast from a fan The dried powder was extracted with cold petroleum ether and saponified after concentration to remove the fatty impurities Crude pigment was precipitated with methyl alcohol from the chloroform solution of the unsaponifiable residue The product was purified by successive re-crystallizations from hot petroleum ether and finally from hot cyclohexane All the processes were carried out in an inert atmosphere to avoid the oxidization of the pigment The melting point of the final product was 172–3°C (uncorrected) Approximately 0 5 g of carotene was obtained from 25 kg of fresh carrot roots

The animals selected for these experiments and the vitamin A-free basal diets used in each case are shown in Table I

Table I

Number	Animal	Number of animals	Vitamin A free basal diet
1	Albino rats*	12	Usual mixture of casein 20, heated rice starch 71, salt mixture 4, dried brewers' yeast 5
2	Cats	2	Boiled lean meat and skimmed milk powder
3	Rabbits	2	Whole grains and bran of wheat and barley
4	Pigeons	2	Husked, unpolished white rice
5	Chicken	2	Wheat, whole grains

* One dozen young albino rats were obtained from Kasauli through the kind courtesy of Sir S R Christophers, then Director of the Central Research Institute

The animals employed for these tests were, as far as possible, young The rats received, in addition to their basal diets, a drop of an olive oil solution of irradiated ergosterol (0 001 mg daily) to supply adequate amounts of vitamin D Other animals received the basal diets alone The animals were kept on their respective

basal diets for an initial period of eight weeks The rats began to decline in weight after six weeks, but other animals showed rather irregular growth curves, presenting on the whole a slight increase in body-weight After eight weeks the animals were divided into two equal groups One group was kept as control and maintained on the basal diet alone, while the other received a carotene supplement at the rate of 0 5 mg per 1 kg of body-weight, daily The carotene was fed dissolved in olive oil and the administration was continued for a period of eight weeks After the elapse of this period the animals were killed and their blood and liver tissues were collected for examination

The tissues were ground up with anhydrous sodium sulphate and extracted with ether The isolated fats were then analysed for their contents of carotene and vitamin A Vitamin A was assayed by a slight modification of the method of Carr and Price (1926) and carotene was determined colorimetrically by comparison with a standard carotene curve made from pure carotene of 184 5°C melting point Lovibond tintometer of the Rosenheim-Schuster type (1927) was used for the estimation of the colour and the vitamin units Total yellow and blue units are expressed according to the scheme adopted by Moore (1930)

The results of analyses are summarized in Table II —

TABLE II

Animal	Number of animals	Average weight of animals (kg)	Carotene given per day (mg)	Name of tissue	Total weight of tissue (g)	Total weight of fat (g)	Total yellow units (Lovibond)	Total carotene found (mg)	Total blue units	Vitamin units per g of tissue
Rat	6	0 125	0 062	Blood	6 4	0 013	0	0	0	0
				Liver	28 0	1 156	124	0 066	150,000	5,357
	6	0 101	0	Blood	8 5	0 018	0	0	0	0
				Liver	25 5	0 937	0	0	0	0
Cat	1	2 79	1 4	Blood	75 5	0 101	3 0	0 0016	0	0
				Liver	61 0	2 583	450	0 241	1,160	19
	1	2 61	0	Blood	106 0	0 078	trace	trace	0	0
				Liver	65 1	1 610	130	0 070	1,740	26

TABLE II—*concld*

Animal	Number of animals	Average weight of animals (kg)	Carotene given per day (mg)	Name of tissue	Total weight of tissue (g)	Total weight of fat (g)	Total yellow units (Lovibond)	Total carotene found (mg)	Total blue units	Vitamin units per g of tissue
Rabbit	1	0 88	0 44	Blood	51 0	0 0025	0	0	0	0
				Liver	39 0	0 959	60	0 032	4,500	115
	1	0 69	0	Blood	20 0	0 0021	0	0	0	0
				Liver	22 0	0 575	30	0 016	330	15
Pigeon	1	0 294	0 147	Blood	13 5	0 004	0	0	0	0
				Liver	6 5	0 190	20	0 01	330	50
	1	0 295	0	Blood	5 0	0 005	0	0	0	0
				Liver	5 5	0 138	12	0 006	trace	trace
Chicken	1	0 47	0 235	Blood	26 0	0 008	trace	trace	0	0
				Liver	17 5	0 494	510	0 270	6,600	377
	1	0 534	0	Blood	42 0	0 008	trace	trace	0	0
				Liver	21 5	0 371	120	0 064	550	25

CONCLUSIONS

The results summarized in Table II appear to suggest that there is a difference in the capacity of different animal species to synthesize vitamin A from carotene On feeding carotene in amounts proportional to the body-weights of animals the rats developed the largest number of vitamin units in their livers There is a significant increase in the number of vitamin units in the livers of all the other animals excepting the cats, but this increase is small in comparison with the vitamin formed by the rat If the capacity for the conversion of carotene into vitamin A is expressed as 100 for the rat, that of the chicken, rabbit, pigeon and cat would be 24, 16, 1 2 and 0 respectively The livers of the control animals also show a certain number of blue units, showing that their reserves of vitamin A were not quite depleted during the four months that they were on vitamin A deficient diet The cat appears to lack altogether the capacity for converting carotene into vitamin A.

A possible explanation of this difference in the vitamin A units formed in the livers of these animals is that vitamin requirements of some animals for their normal physiological functions are so large that stores of vitamin A cannot develop in their livers as readily as in those of others This, however, is unlikely In some of the unpublished experiments by one of us (B A) it was found that while no vitamin A developed in the livers of cats fed with as much as 10 mg of carotene per day for 12½ weeks (200 times the equivalent of a normal rat dose), the feeding of relatively small amounts of liver oil concentrates (twice the equivalent of a normal rat dose) resulted in the accumulation of a store of vitamin A in the liver It appears therefore that differences of vitamin units found in the livers of animals, as set down in Table II, are not to be explained on differences of normal vitamin requirements, but on differences of their capacity to utilize carotene for vitamin A formation The observations of Capper, Mckibbon, and Prentice (1931) * that the minimum daily requirements of the chicken for carotene are almost 12 times larger than the rat, are also to be explained in a similar way

The inability of the cat to form vitamin A from carotene has been repeatedly observed by us during many experiments Recently Rea and Drummond (1932) have also stated that no vitamin A is formed by cat's liver from carotene Euler and Euler (1931) have observed that the addition of carotene to the diet of fish did not result in its conversion to vitamin A It appears therefore that carnivorous animals to whom a ready-made supply of vitamin A is always assured through food derived from animal sources have lost the function of synthesizing vitamin A from carotene If this be so man would also fall into this category

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A NOTE ON THE SUN DISINFESTATION OF GRAINS AS A MEASURE IN THE PREVENTION OF PLAGUE

BY

C G PANDIT, PH D, M B, B S, D P H, D T M,
K P MENON, L M & S, L R C P & S,

AND

P V SEETHARAMA IYER, M A
(*From the King Institute, Guindy*)

[Received for publication, November 22, 1932]

THE sun disinfestation of grain has been advocated as a means to prevent the introduction of infected fleas, etc, into a locality and orders to treat grains in this way have been passed by some Governments as part of their plague preventive measures No definite rules have been laid down either as to time of the day when the grain is to be so disinfested, or the manner in which to do it It is obviously impracticable with many cart-loads of grain to empty it out of the gunny bag, disinfest it and then re-bag it The best that can be done is to expose the loaded gunny bags to the action of the sun There are, however, considerable practical difficulties even in this procedure In order, therefore, to study the limitations of this method, we decided to make some observations on the degree of penetration of heat into the varying depths of the grain in gunny bags, when such bags were exposed under the most favourable conditions to direct sunlight

These observations were made in 1929 in May and June—the hottest period in the year in Madras

A cement platform was constructed in the most exposed part of the Institute grounds The gunny bags containing grains were placed in the platform at 10 A M and allowed to remain there till 4 P M A thermometer was placed on the top of the gunny bag T_0 just inside and touching the gunny, T_1, T_2, T_3, T_4, at successive depths of about 4 inches until the bottom was reached, the last being at the bottom of the grain The temperatures were recorded every hour from 10 A M

to 4 P M The following were the varieties of grains so exposed ragi, Bengal gram, rice, bran, green dhall, horse gram, black gram, and dhall The following is the summary of our observations —

1 The maximum temperature with each grain was recorded by the thermometer placed just underneath the gunny The maximum reading in different days varied between 40°C and 66°C , air shade temperature varied from 33 9°C to 40 9°C

2 The maximum temperatures obtained with any grain with the thermometers at various depths within the grain varied from 33°C to 43°C

3 The daily maximum temperature recorded was always between the hours of 12-30 and 2-30 P M

4 Sudden falls in the temperatures were observed with changes in the velocity and the direction of the wind

5 The heat-receiving power of different grains varied, e g , bran and rice which were bagged in similar kind of gunnies and exposed to the sun on the same day under identical conditions gave the following centigrade temperature readings —

Rice							Bran						
Time	T_0	T_1	T	T_3	T_4	Shade	Time	T_0	T_1	T_2	T_3	T_4	Shade
11-30	55	56	33	33	34	38 9	11-30	55	65	35	34	39	38 9
12-30	57	59	33	33	34	39 7	12-30	57	66	35	34	39	39 7
1-30	52	55	34	33	35	37 8	1-30	52	63	36	34	39	37 8
2-30 .	47	46	34	34	35	37 5	2-30	47	56	36	35	40	37 5
3-30 ..	44	43	35	35	35	37 2	3-30	44	54	38	35	40	37 2

Similarly, as regards black gram and dhall it was found that when both these were exposed to the sun on the same day, the maximum temperature developed in the gunny containing black gram was found to be 57°C , whereas dhall developed a temperature of only 56°C Similarly, when the maximum for ragi was 58°C , that for Bengal gram was only 52°C on the same day

As noted by Cunningham (1911) a temperature of 49°C for 45 minutes is necessary to kill all fleas Since this temperature is only obtained on the surface

and just below the gunny, this method of disinfestation would only be effective on the topmost layer of grain next to the surface of the gunny that is exposed to the sun

A few experiments were then conducted to determine to what depth fleas or their larvæ penetrate into bagged grains (1) under conditions obtaining in godowns and (2) when the bagged grain was exposed to the action of sunlight The grains stocked in godowns, however, are not always kept in bags, but are usually measured and bagged only at the time of transport The loose fleas and then larvæ found in godowns are, therefore, likely to be mixed up among the grains and well distributed in the bags at all depths

The first set of observations were made to elucidate the following points —

1 Whether adult fleas or their larvæ can penetrate through gunny and live in grain stored in gunny bags

2 The depth from the surface of the gunny bag to which they penetrate when introduced outside the bag and left at room temperature

3 The period of time the adults or their larvæ can live in such surroundings

4 Whether any particular grain affords better facilities for the conveyance of fleas from one place to another

Technique of the experiment —As designed by Lieut -Colonel King, rectangular boxes of deal-wood were made One end of each box was covered with a tightly-stretched gunny, while the other end was closed with a movable lid The whole box could be divided into compartments by means of removable partitions one inch apart The partitions were of thin tin plates which could be inserted when required without disturbing the grain The experiment consisted in filling the box with a grain after having removed all the intermediate partitions Bits of dried blood-soaked rag or rat fæces were placed between the meshes of the gunny A bag of muslin containing a sufficient number of fleas which had fed on rats was tied over the gunny These were *X astia* obtained from local rats The whole box was left in a well-shaded room, the temperature of which for the period (August-September 1929) varied from 28 2°C to 32 6°C The wet bulb reading for the same period ranged between 25°C and 26 5°C Just before each box was examined, the movable partitions were closed in so as to divide off compartments in the grain

Two experiments with bran and one with rice were carried out each with five boxes

The first four boxes were examined after 1, 2, 3 and 4 days respectively Since all adult fleas were found to be dead in the last two boxes, the fifth or remaining

box was left for a fortnight to see whether larvæ survived In each box the contents of each compartment were examined in turn for live or dead fleas or then larvæ A set of sieves of different grades was found useful in searching for larvæ

Results

1 Adult fleas (*X astia*) were found to live not more than three days under experimental conditions during this season

2 Fleas were found to have penetrated through the gunny (bran experiment) but they were all found dead in the first compartment adjacent to the gunny Most of the fleas were, however, lying dead on the surface of the gunny itself

3 Two larvæ were obtained alive after 16 days in the compartment adjacent to the gunny (bran experiment)

4 The box containing rice did not show any flea or larvæ inside The presence of mites in large numbers in rice is a factor to be considered in this experiment These are said to destroy flea larvæ They were certainly present on this occasion

These experiments suggest that —

1 When bags are kept in the shade fleas and their larvæ exhibit a tendency to remain in or move into that portion of the grain in the gunny bags near the surface, for the adult fleas and then larvæ were always found in the first compartment, i e , at a depth not greater than one inch from the surface of the bag

2 While the average life of adult unfed *cheopis* is stated to be only a little longer than that of *astia* (Webster, 1930) this observation rather suggests that the transportation of infected fleas is unlikely when the journey is of several days' duration

3 The duration of life of the larvæ under the same conditions is more than 16 days This suggests the possibility of the introduction of fleas in their larval stage from one locality to another

The fleas and their larvæ in these experiments were introduced outside the gunny bag and kept in a room with a temperature range of only 28 2°C to 32 6°C Since in the sun disinfestation of grains in gunny bags, the maximum temperature is obtained on the surface of the gunny and the layer of grain just below it, it was considered possible that this method of disinfestation might result in the destruction of all fleas and then larvæ, if these continue to remain near the surface or try to escape outside from the bag when exposed to the action of the sun

At Lieut -Colonel King's suggestion two further experiments were designed and carried out by the second and third authors (K P M and P V S) to test whether this inference was correct

EXPERIMENT I

Live fleas (*X astia*) removed from a rat and flea larvæ were mixed up with rice bran and introduced into the experimental box, the partitions having been removed previously The box was put out in the sun during the hottest part of the day for three hours The partitions were wedged in and each compartment was examined to see whether fleas and the larvæ were dead or alive and in what compartment they were numerous

Results

1 The largest proportion of fleas was found collected in the third compartment, 2 to 3 inches from the surface

2 The fleas collected were all dead except two The two live fleas were found in compartment III

3 No living or dead larvæ could be detected—probably all larvæ were dead and could not be detected

EXPERIMENT II

Live fleas (*X astia*) immediately after removal from rats and flea larvæ were introduced on the inside surface of the gunny in the experimental box which was then filled with rice bran The box was put out in the sun, gunny uppermost for two hours The partitions were wedged in and each compartment was examined in turn

Results

1 Fleas found in the compartment adjacent to the gunny and on the gunny were dead

2 Live fleas were found in compartments II, III and IV

3 Living larvæ were also obtained in compartment III

4 Of the 30 fleas recovered, 11 were from compartment II, and 9 from compartment III, of which 4 and 6 were alive respectively In compartment I at the top, 9 dead fleas were found and in IV at the bottom which is usually as hot as the top, one live flea was found

These experiments suggest that even if fleas tend to congregate on the peripheral layers of grain in bags under normal conditions, *they can and do actively migrate into the interior of bags when exposed to the action of the sun* They tend to remain in that portion of the bag where the temperature conditions are the lowest

According to Cunningham (*loc cit*) a temperature of 49°C for 45 minutes is essential for the destruction of fleas It would, therefore, appear that even if sun disinfestation can be conducted at the most favourable time, i e, between 12-30 and 2-30 P M, it would not be possible to obtain this critical temperature *on all days* and as the temperatures in the interior of gunny bags had always been lower than the critical one, even under favourable conditions, it would not be possible to have the entire grain so disinfested

It must be remembered that the necessity for the disinfestation will usually arise during the plague season, i e, in the monsoons or the cold weather when it is certain that the critical temperature required would never be obtained We therefore conclude that sun disinfestation as a practical preventive measure is of no value in the climate of South India

Acknowledgment—We are indebted to Lieut-Colonel H H King, I M S, the Director of the Institute, for his suggestions and advice in the course of this work

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Ind Jour Med Res, XX, 4, April, 1933

THE VITAMIN CONTENT OF THE INDIAN MANGO

BY

B C GUHA, PH D D SC (London),

AND

P N CHAKRAVORTY, B SC

(From the Biochemical Laboratory, Bengal Chemical and Pharmaceutical Works, Ltd, Calcutta)

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ALTHOUGH a large number of the commoner food-stuffs used throughout the world have been investigated with reference to their vitamin potency, some of the relatively important Indian food materials still remain to be investigated In recent years some of the older methods of assay have been revised especially with regard to 'vitamin B' The recent researches of Goldberger *et al* (1926, and other papers) have left little doubt that the so-called 'vitamin B' contained at least two factors B_1 (the anti-neuritic vitamin) and B_2 (the anti-pellagra vitamin), the absence of which from the diet produced quite distinct pathological symptoms in the experimental animals Following on these important researches additional evidence has been presented that vitamins B_1 and B_2 are physiologically and chemically quite unlike (Guha, 1931, 1931*a*) and methods of biological assay for these two factors have been described (Guha and Drummond, 1929, Guha, 1931, 1931*a*) These new facts have, in several countries, led to a necessary re-investigation of a series of food-stuffs which were originally assayed for 'vitamin B'

In the present paper experiments on the content of vitamins A, B_1, B_2, and C in the Indian mango are described The three chief types of mango known in Bengal as the 'Langra', 'Bombai' and 'Fozli' were investigated As this work was in progress, a short report was published of the experiments of Perry and Zilva (1932) on the vitamin A, C and D content of the 'Alphonso', 'Cowasji Patel' and 'Shendrya' varieties of the Indian mango They do not appear to have investigated the vitamin B_1 and B_2 content A comparative estimate of our results and of Perry and Zilva's indicates that, while in some respects the different varieties of the Indian mango have general resemblance to one another in their vitamin potency, there are certain differences among the several varieties in a few respects These results are discussed later

EXPERIMENTAL

(*a*) *The assay of vitamin A* —The methods of assay of vitamin A have been discussed extensively in recent years (Drummond and Morton, 1929, Ahmad and

Drummond, 1930, Coward and Key, 1928) The colorimetric method originally devised by Rosenheim and Drummond (1925) and modified by Carr and Price (1926) appears to be a reliable method for the estimation of vitamin A, at least so far as the cod-liver oils are concerned The activity of plant products appears, however, to be due not so much to the presence of the 'classical' vitamin A of liver oils but of its precursor, the lipochrome pigment carotene, which has been shown to be converted into vitamin A in the body (Euler, Euler, and Hellstrom, 1928, Euler, Euler, and Karrer, 1929, Moore, 1929, 1930, Collison *et al*, 1929) The antimony trichloride colour reaction of carotene is different from that given by the 'classical' vitamin A and the coloured solutions produced in the two tests have also different absorption spectra In the assay of plant products for vitamin A, it, therefore, appears desirable to adhere to the biological method

Young albino rats of about 50 g weight, which were kept in separate cages with screened bottoms, were placed on the following basal diet —

Diet (5)—Vitamin A—deficient basal diet

Cassava starch (baked in the air oven at 105°C for 4 hours)	75 parts
Casein (B D H 'light, white')	20 ,,
Dried yeast	5 ,,
Salt mixture (McCollum)	5 ,,

Each of the rats received in addition one drop of Radiostol (B D H) on every Tuesday and Friday, to serve as a source of vitamin D The rats began to decline on this ration after a period of 4 to 5 weeks The mango pulp to be tested for vitamin A was then fed to the animals at different levels A consequent gain in weight of about 10 g for 2 weeks was taken as a standard of good growth

(*b*) *The assay of vitamins B_1 and B_2* —Young albino rats of about 50 g weight were fed on a basal diet of the following composition —

Cassava starch	75 parts
Casein (B D H 'light, white')	20 ,
Salt mixture (McCollum)	5 ,

Each of the animals received separately in a dish 1 to 2 drops of a cod-liver oil of proved potency for vitamins A and D The animals to be used for B_1-tests were also given throughout a preparation of liver extract as a source of vitamin B_2 (Guha, 1931*a*) made by extracting 700 g of fresh buffalo-liver with 1,166 c c of boiling water for 4 minutes The clear brown filtrate, while it was rich in vitamin B_2, contained only traces of vitamin B_1 When the animals began to decline on this dietary, they were fed on the mango pulp at different levels A weekly increase in weight of 10 g lasting for 2 to 3 weeks was taken as the standard of good growth

The animals to be used for vitamin B_2-tests received in addition to the basal diet and cod-liver oil a preparation obtained from rice-polishings in the following manner 1 kg of rice-polishings was extracted with 3 litres of boiling water, acidified with 6 c c concentrated HCl (Merck's) for 6 minutes and filtered under suction

The filtrate was treated with a saturated solution of neutral lead acetate just sufficient for complete precipitation After the removal of the lead precipitate, the filtrate was freed from lead with hydrogen sulphide and finally from hydrogen sulphide by concentration under reduced pressure This was practically free from vitamin B_2 The subsequent technique was the same as that used for the assay of vitamin B_1 That the extracts of liver and rice-polishings were very potent preparations of vitamins B_2 and B_1 respectively is shown by the fact that they together sustained good growth for several weeks of the young rats fed on the diet deficient in the vitamin B-complex although neither, fed by itself, could maintain growth

(*c*) *Biological assay of vitamin C* —Guinea-pigs of 250 g to 300 g weights were fed on a basal diet of oats (85 g) and bran (15 g) with which 1 per cent of cod-liver oil was incorporated In the course of two weeks they began to decline in weight The effect of mango pulp fed at different levels, compared with that of lemon juice, on the growth curves of these animals was observed Animals receiving neither supplement died with severe scorbutic symptoms Autopsy revealed extensive hæmorrhages in the knee-joints and gums

Preparation of the mango pulp

A few mangoes of each variety were peeled and the pulp, scraped from the stones, was thoroughly disintegrated and mixed, and subsequently strained through muslin in order to remove fibrous material The thick liquid was then preserved in amber-coloured stoppered bottles at 0°C while being fed

Results

The variety of mango	Dose (c c)	* Average weekly growth (in g) produced by vitamin			
		A	(B_1+B_2)	B_1	B_2
'Bombai'	3			4	10
	3		1		10
	5		10		
	6		13 3		
'Langra'	3		2	4	
	5	6	2	4	
	7	14		11	
'Fozli'	3			10	
	7	15		9 5	
	10	28			

* Average weekly growth represents the average growth response of several animals to a particular dose

Vitamin C.—'Bombai' mango pulp was assayed for its vitamin C content at levels of 2 and 3 c c daily dose only The guinea-pigs died within 2 to 3 weeks with severe symptoms of scurvy Higher doses were not tried

Discussion and summary

A comparison of our results with the preliminary report published by Perry and Zilva (*loc cit*) indicates that there are substantial variations among the different varieties of the Indian mango So far as the vitamin A content is concerned, all the varieties appear to be potent sources of this factor The varieties, we have tested, are fairly good sources of the 'vitamin B-complex' The 'Bombai' mango is, however, much richer in vitamin B-complex than either 'Langra' or 'Fozli' 'Bombai' is apparently richer in B_2 than in B_1 The other varieties were not tested directly for vitamin B_2 The vitamin C content of the 'Langra' variety is not of the same order as that of lemon juice, while the varieties tested by Perry and Zilva are stated to be highly potent sources of vitamin C This work suggests the desirability of the investigation of all the varieties of a particular fruit, as they are likely to differ materially from one another in their vitamin content

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Ind Jour Med Res, XX, 4, April, 1933

A NOTE ON THE FINDING OF *TOXOPLASMA CUNICULI* IN TWO EXPERIMENTAL RABBITS

BY

K V KRISHNAN, M B, M R C P E, D B (London), D Sc,

AND

JEMADAR CHIRANJI LAL, I M D

(From the Kala-azar Inquiry, Indian Research Fund Association, School of Tropical Medicine, Calcutta)

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ORGANISMS included in the genus *Toxoplasma* have been found in the blood and tissues of a number of different vertebrates in several parts of the world *Toxoplasma cuniculi* in the rabbit was first described by Splendore (1910) in Brazil, subsequently by Bourret (1911) in Senegal and by Brug, den Heyer, and Haga (1925) in Dutch East Indies As far as we are aware these organisms have not been reported from India Adie (1908) described a parasite in the Indian sparrow which Wenyon (1926) thinks may be toxoplasma In the course of certain experiments to 'blockade' the reticulo-endothelial system in the rabbit, we encountered this parasite in two out of twelve animals In these animals toxoplasma was apparently the cause of a severe fatal illness, characterized by progressive loss of weight, diarrhœa and anæmia As the circumstances under which these parasites were found are peculiar, we decided to publish this brief note about them

The two rabbits that developed toxoplasma infection belonged to a batch of 12 experimental rabbits that had received first a course of 15 injections of Indian ink and colloidal iron, followed by three intravenous injections of a culture of *Leishmania donovani* Eight weeks after the last injection the two rabbits began to look sickly and to lose weight They would not feed properly and were passing loose motions Repeated examinations of their stools revealed no Coccidia or other infection to account for the diarrhœa Peripheral blood examinations showed a high reticulocyte count (20 per cent) and a low leucocyte count together with an increase in the

number of large mononuclear cells On further examination of the blood of one of these the presence of Leishmania-like bodies within a few large mononuclear cells was detected Suspecting that we had succeeded in giving this rabbit leishmania infection we decided to sacrifice it Post-mortem examination revealed a large number of tiny necrotic foci in the spleen and liver Smears and cultures in N N N medium, from the spleen, liver, bone-marrow and heart blood, were made Large numbers of toxoplasma were found in every smear examined, but no growth of any organism was obtained in any of the N N N tubes inoculated

The second rabbit, which was being kept under observation, was found dead one day about three weeks later, and toxoplasma were found in the smears from liver and spleen As decomposition had set in before the post-mortem examination could be performed, no attempts were made to transmit the infection to other animals for further study

The different forms of the parasite met with in the smears are given in Plate XLV Both intra-cellular and extra-cellular forms were seen, and they did not in any way differ from the textbook description of the organism

The points of special interest about this finding are —

that it is now shown that toxoplasma infection in rabbit does occur in India,

that such infection appears to be the cause of severe illness and death in these animals,

that toxoplasma within large mononuclear cells may at times be mistaken for *L donovani*,

that blockade of the reticulo-endothelial system by injections of colloidal iron and Indian ink can, by lowering the resistance of the rabbits, probably cause a flare up of latent toxoplasma infections

Our thanks are due to Lieut -Colonel R Knowles, I M S , Professor of Protozoology, School of Tropical Medicine, for helping in the identification of the organism

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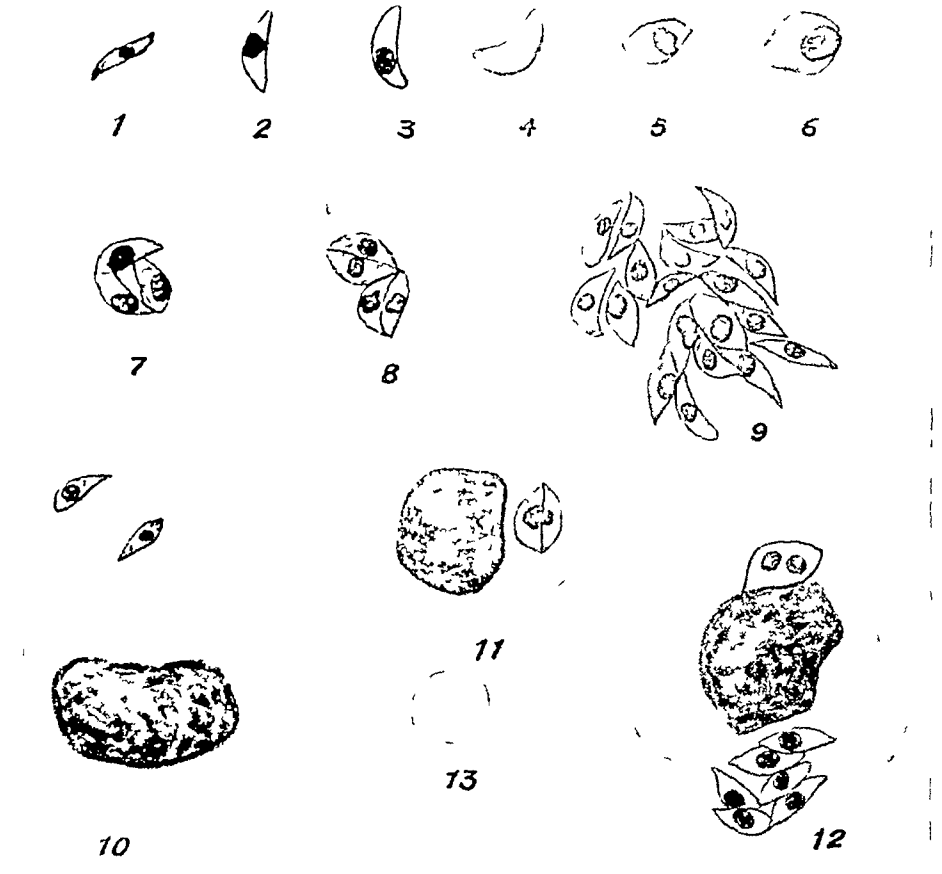
Plate XLV

Toxoplasma cuniculi.



(The figures in the plate represent camera lucida drawings of toxoplasma as seen in smears fixed and stained with Giemsa's stain)

Figs 1 to 6 Different forms of the parasite seen—Note great variation in size and shape
Fig 7 A small group of parasites
„ 8 Dividing forms—longitudinal division
„ 9 A large cluster of parasites
„ 10 A large mononuclear cell from the peripheral blood showing two parasites which resembled somewhat *Leishmania donovani*
, 11 A large mononuclear cell from the spleen smear showing a dividing form within a vacuole
, 12 A large mononuclear cell from spleen smear showing a dividing form and a group of parasites
, 13 A red blood corpuscle for comparison of size

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A NOTE ON THE REVERSIBLE OXIDATION REDUCTION OF GLUTATHIONE IN LIVER

BY

KSHITISH CHANDRA SEN, D Sc ,

Biochemist,

Imperial Institute of Veterinary Research, Muktesar

[Received for publication, December 2, 1932]

SINCE the discovery of glutathione by Hopkins (1921) a large amount of literature has grown up concerning the rôle of this substance in physiological processes It was found by Hopkins that the SH group of glutathione could be easily oxidized and again reduced, and Hopkins and Dixon (1922) showed that muscle tissue, whether intact or thoroughly extracted with hot water, reduced the oxidized form of glutathione with ease, and the reduced form could again be oxidized by molecular oxygen In tissue oxidation therefore the function of glutathione was as a carrier of hydrogen from suitable donators to molecular oxygen by virtue of its sulph-hydryl groupings The position, however, became complicated when Meldrum and Dixon (1930), studying the properties of pure crystalline glutathione, found that glutathione was not auto-oxidizable, though the oxidized form could be reduced by tissues Impure glutathione was, as has been previously observed, auto-oxidizable, and the difference was found to be due probably to a metal and cysteine or cysteinylglycine complex present in the impure specimens Doubts therefore arose as to the true function of glutathione, because in *in vitro* experiments the pure substance failed to show any uptake of oxygen in presence of washed tissue In view of this stability of the pure substance towards oxygen Hopkins and Elliot (1931) thought it desirable to study the reactivity of glutathione when present in fresh intact tissues and to follow its fate during the course of survival respiration It was found by them that the reduced form of glutathione in normal hepatic tissue was readily oxidizable to the disulphide form by molecular oxygen, and that the oxidation process was always initially associated with a reduction process, showing that the transport of hydrogen to oxygen is maintained, and indicating that the oxidation of the glutathione has real metabolic significance

An important point to be considered in all glutathione studies is the fact that in practically all living tissues glutathione exists mostly in its reduced form and though it must be assumed from Hopkins and Elliot's work (*loc cit*) that it is being continually oxidized by molecular oxygen when present in its natural surroundings, at least in the liver-tissue, a continuous reduction process must also be going on simultaneously and at any particular moment there would be a dynamic equilibrium between the two forms of glutathione It is, however, not known what are the agencies which keep the glutathione reduced in the tissues From the earlier work of Hopkins and Dixon it was presumed that this reduction may be due to the fixed SH group of the thermostable tissue, but Hopkins and Elliot (*loc cit*) have found that liver-tissue heated to 50°C loses much of its reducing power, thus indicating that there is some thermolabile reducing system involved in the process It was therefore thought necessary to make a further study of this aspect of the problem, and at the suggestion of Professor Hopkins an attempt was made to find out the nature of the reducing systems As many of the experiments did not give any clear-cut result they will be mentioned only briefly later on, and as the present position of the subject is still in an unsatisfactory state, a short discussion will be given on the theoretical aspects of the problem

Experimental technique

The methods for the estimation of glutathione in tissues are not yet very satisfactory, but good duplicates were obtained by using Kuhnau's (1931) procedure of adding 20 c c of 25 per cent potassium iodide to the solution of the thiol compound before the iodine solution was run in The glutathione was extracted from the liver-tissue by coagulating the protein with 10 per cent trichloracetic acid, grinding it with sand in a mortar and filtering under suction The residue was again ground up with further addition of trichloracetic acid and filtered This process was repeated thrice, and the total thiol content of the combined extract was oxidized by means of excess of N/100 iodine and the excess then determined by thiosulphate with starch as the internal indicator The total amount of iodine used up was considered to be equivalent to the whole of the reduced glutathione (*cf* Hopkins and Elliot, *loc cit*) In the majority of the experiments given below, fresh rabbit liver was used The tissue was dried within filter-papers and then thoroughly chopped up by means of a pair of scissors This method did not break up the cells and gave quite uniform results Known weight of the tissue was taken in bottles or in tubes which could be evacuated (depending on the nature of the experiment, aerobic or anaerobic), and mixed with a certain amount of Ringer's solution containing bicarbonate, suitable for mammalian tissues, and then the substance or substances whose effect was being studied were added The pH of the final mixture was kept at 7 6 The bottles or tubes were then shaken vigorously in a shaker for different periods of time at room temperature, after which the glutathione

content was estimated A drop or two of chloroform was added to prevent bacterial growth A blank determination before the start of the experiment gave the original amount of glutathione in the sample

THE NATURE OF THE THERMOLABILE REDUCING SYSTEM IN LIVER

A number of investigators[c] have previously tried to reduce oxidized glutathione by means of a specific dehydrogenase and its substrate, for example succinic dehydrogenase, xanthine oxidase, etc (Hopkins and Dixon, 1922, Elliot, 1928), but the positive results of Wieland and Bergel (1924) with succinate have not been substantiated The results obtained by me with substrates like succinate, lactate, etc, also gave similar negative results, and when chopped liver-tissue was kept in aerobic incubation with the addition of these substrates there was no indication that a higher reduction of disulphide was obtained as compared with the control The presence of glucose, however, sometimes gave slightly positive indications Thus the following details will give the results of a few experiments with glucose, succinate, and lactate, and incidentally will show the procedure adopted for the experimentation —

Ten grammes of chopped rabbit liver were placed in wide-mouthed bottles and 20 c c of bicarbonate-Ringer's solution were added to each Two bottles served as controls, while in others, glucose, sodium succinate, or sodium lactate was added to make the concentration 2 5 per cent with regard to the added substance The pH was adjusted, the bottles were tightly stoppered by means of rubber-stoppers, leaving a sufficient air space, two drops of chloroform were added and then the bottles were shaken in a motor-driven shaker for four hours After this period, trichloracetic acid was added to all the bottles and the total thiol content in each was estimated in the way stated above The results of three such experiments are shown in Table I —

TABLE I

Contents of bottle	Initial amount of SH in terms of I. N/100 c c	Final amount of SH in terms of I_2	Amount of SH oxidized
Experiment I			
(*a*) Pure liver	10 22	6 8	3 45
(*b*) Liver + succinate	10 25	7 1	3 15
(*c*) Liver + glucose	10 25	6 6	3 65
Experiment II			
(*a*) Pure liver	11 0	8 55	2 45
(*b*) Liver + lactate	11 0	8 23	2 77
Experiment III			
(*a*) Pure liver (8 grammes)	9 1	2 15	6 95
(*b*) Liver + glucose	9 1	2 96	6 14

The different experiments were carried out with the livers of different animals It will be observed from the results of experiments I and II given in Table I that the differences in the amount of oxidation of SH in presence or absence of succinate, lactate or glucose in four hours were not marked, and as the error in the analytical method may go up to 10 per cent, no definite acceleration of the reducing process can be assumed from these results These experiments, therefore, support the conclusions arrived at by previous authors Glucose, however, sometimes gave a fluctuating result as the following experiment with sheep liver will show The experiment was made in a

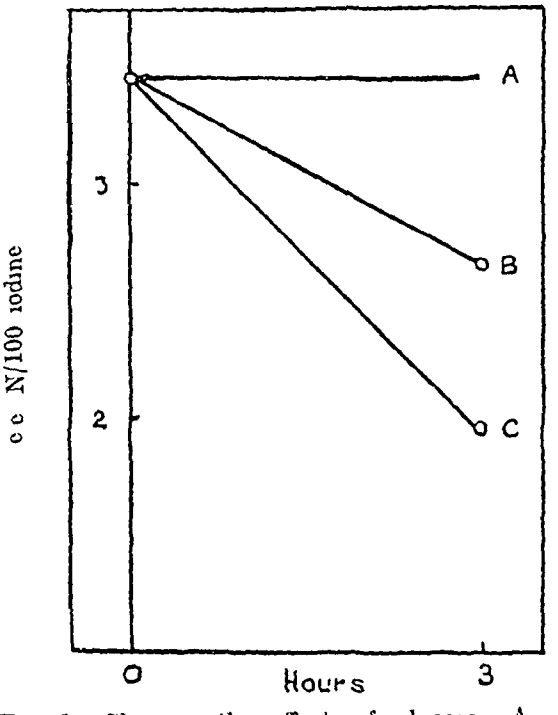

FIG 1.—Showing the effect of glucose A—initial amount of SH, B—amount of SH of liver + glucose, and C—SH of control tissue after 3 hours

way similar to that given above, 8 grammes of chopped sheep liver being used instead of fresh rabbit liver The initial amount of SH was equivalent to 3 45 c c I_2 N/100, the amount of SH after 3 hours of aerobic shaking was equivalent to 1 95 c c I_2, whereas the amount of SH in presence of 2 5 per cent glucose (ordinary Ringer's solution was used) after 3 hours was equivalent to 2 65 c c I_2 The difference between the control and the glucose experiment was about 35 per cent, showing that under some circumstances glucose could be activated so as to supply hydrogen to the reducing mechanism so that more reduction occurred

in liver in its presence during survival respiration This result is shown in Figure 1 (*see* also Experiment III, Table I)

Reduction of added oxidized glutathione

Hopkins and Elliot (*loc cit*) have already shown that oxidized glutathione (SS form), if added to liver-tissue, can be reduced to the SH form even in presence of molecular oxygen In order to find out how far the results can be duplicated, the following experiment was carried out To 10 grammes of sheep liver (16 hours old) in different bottles 40 mg of oxidized glutathione, dissolved in dilute alkali and pH adjusted, were added and the bottles were aerobically shaken along with controls for different periods of time The pure oxidized glutathione was obtained according to the method of Pirie (1931) Reduced glutathione was prepared from yeast (Pirie, 1930) and this was then oxidized by means of hydrogen peroxide and the oxidized glutathione was then crystallized Table II gives the result of this experiment —

Table II

Contents	Initial SH in terms of I	Final SH in terms of I	
		½ hour shaken	4 hours shaken
(*a*) Pure liver	11 0	9 4	5 1
(*b*) Liver and oxidized glutathione	11 0	11 5	7 0

In Figure 2 the data of Table II are graphically represented It will be observed that during the initial period, the rate of reduction by the reducing system of the liver is much greater than the oxidation of SH by molecular oxygen and hence there is a rise in the SH content of the system The oxidative mechanism, however, gets the upperhand after some time, probably due to the exhaustion of some metabolite, and after four hours of aerobic shaking there is a fall in the total SH content, though even after that period, the total SH of the system containing oxidized glutathione remains higher than that containing no added disulphide

The form of the curves is quite similar to that obtained by Hopkins and Elliot and all these results were obtained before the publication of their paper

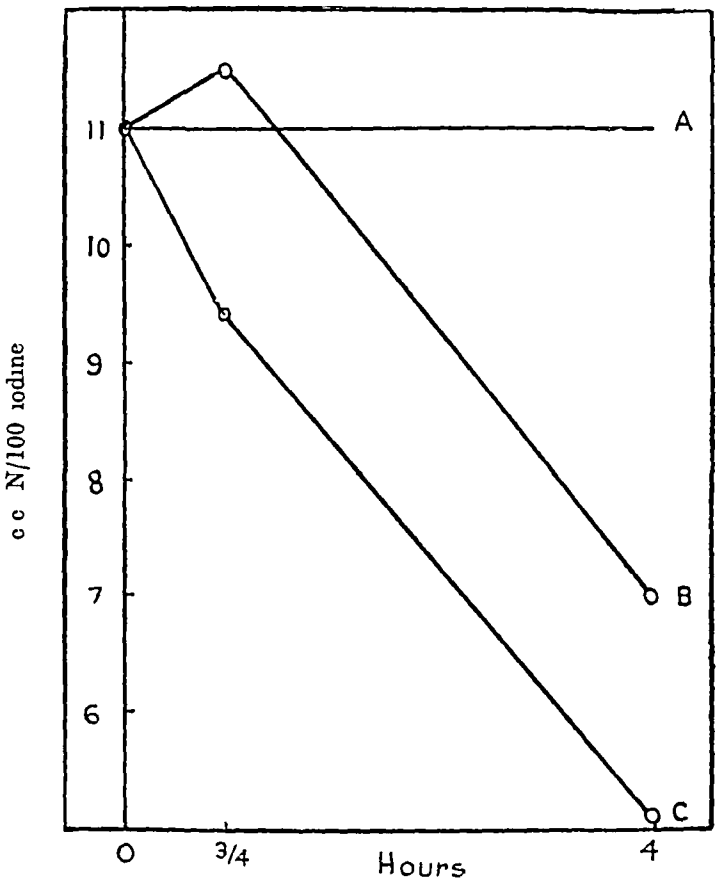

FIG 2 —Showing the effect of addition of SS A—initial SH, B—liver + SS, C—liver, control

EFFECT OF URETHANE

In a recent paper (Sen, 1931) the author has studied the effect of some narcotics on dehydrogenases and has found that narcotics have an effect on systems which act in conjunction with Keilin's cytochrome system In order to find out the nature of the reducing system in liver, it was therefore thought desirable to make some experiments on the effect of narcotics on the oxidation and reduction of glutathione in liver Two series of experiments have been done one—aerobic, and the other—anaerobic In anaerobic experiments where the oxidative factor is dormant, Hopkins and Elliot have already shown that the SH content gradually increases due to the reduction of preformed oxidized glutathione It is therefore not necessary to add any oxidized glutathione to such a system to make the reducing mechanism evident The experiment was therefore arranged simply by adding a certain amount of narcotic to chopped liver-tissue placed in a Thunberg tube, adding the usual amount of bicarbonate-Ringer's solution, and then evacuating it Along

with controls these tubes were then shaken in the usual way and after the experimental period, these were opened under 10 per cent trichloracetic acid solution In Table III, 10 grammes of rabbit liver and 5 per cent ethyl urethane were used —

TABLE III

Anaerobic experiment

Contents	Initial SH	Final SH	
		After 2 hours	After 4 hours
(*a*) Pure liver	7 35	10 4	11 2
(*b*) Liver + urethane	7 35	9 5	9 3

Figure 3 shows the results of Table III It will be observed that the reduction of some preformed SH goes on rapidly on anaerobic incubation of liver-tissue and

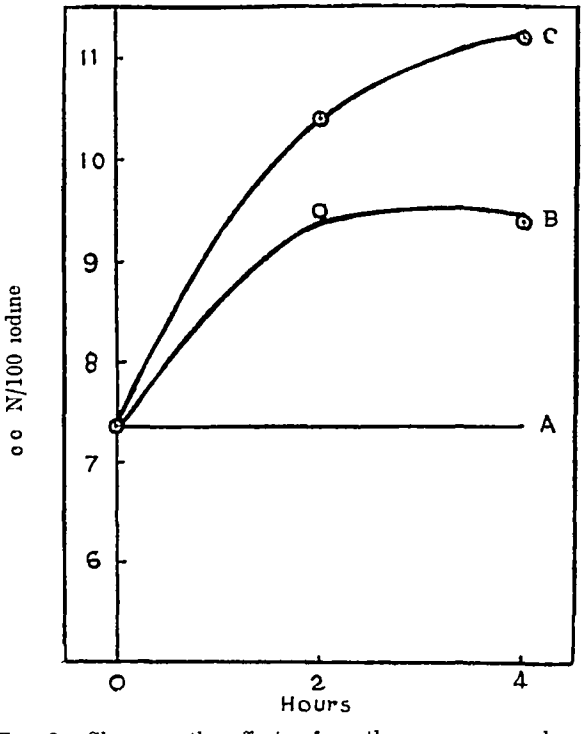

FIG 3 —Showing the effect of urethane on anaerobic reduction of SS A—initial SH, B—liver + urethane, C—pure liver, control

hence the reducing mechanism is fairly active It will also be noticed that 5 per cent urethane diminishes this reducing action considerably and after four hours'

standing, the difference between the total SH content of the control and narcotized liver is about 28 per cent of the initial SH In other words, the inhibition of the reducing mechanism is about 28 per cent In Table IV the effect of 5 per cent urethane on the aerobic reduction of added oxidized glutathione by liver-tissue is shown Ten grammes of rabbit liver and 40 mg of disulphide were used

TABLE IV

Contents	Initial SH	Final SH after 4 hours
(*a*) Liver + disulphide	7 4	11 75
(*b*) Liver + disulphide + urethane	7 4	8 55

The data in Table IV are shown in Figure 4 It will be observed that in the case of aerobic experiments also urethane has a definite effect on the mechanism

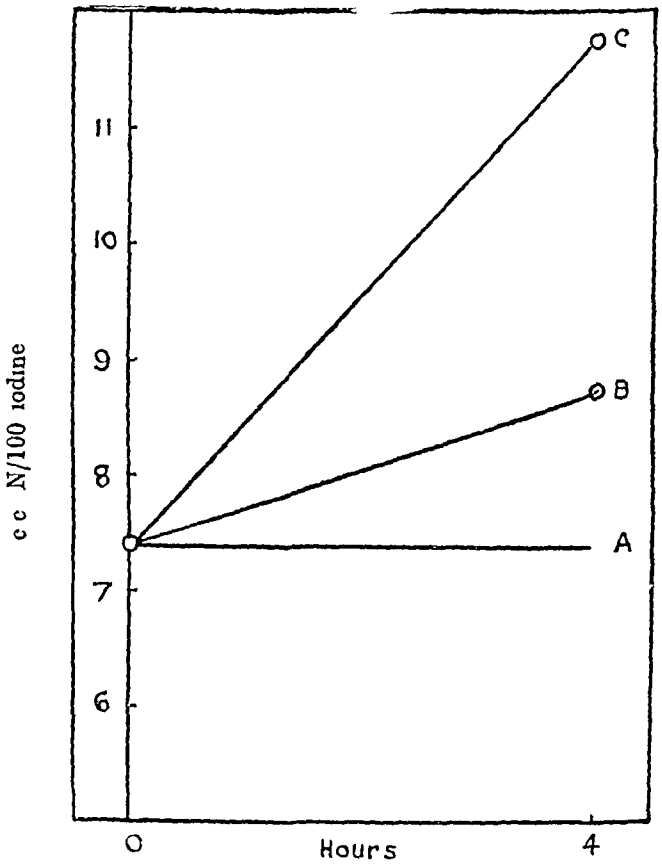

FIG 4 —Showing the effect of urethane on the aerobic reduction of added SS A—initial SH, B—liver + urethane + SS, C—liver + SS, control

responsible for the reducing action of liver on the oxidized glutathione These results, therefore, raise the question of the nature of the reducing system in the liver-tissue

Discussion

It has already been stated that the thermostable tissue residue reduces oxidized glutathione to a certain extent and, besides this, some thermolabile system is also concerned in the phenomenon Though no dehydrogenase system was known to reduce oxidized glutathione when this work was done, it has recently been found by Mann (1932) that an enzymatic system, similar to that of Harrison's glucose dehydrogenase (1931), can be obtained from liver in a cell-free condition which gives a slight reduction of oxidized glutathione when incubated along with glucose The varying results obtained by me when liver-tissue was incubated with glucose are thus explained This reduction is not, however, very great and it is still an open question as to what is the proportion of reduction by this system compared with the total reduction by the whole of the thermolabile system The effect of some other substances, such as urethane, brings in other difficulties Hopkins and Elliot found that the rate of oxidation of SH is not at all inhibited by carbon monoxide and as such the oxidative mechanism is not related to Keilin's oxidase-cytochrome system or Warburg's 'Respiratory Enzyme' In fact, as the oxidative reaction goes on equally well when liver-tissue is heated to 70°C , Hopkins and Elliot believe that the oxidative process is not enzymatic at all, though it is cyanide sensitive, and hence the oxidation catalyst is probably iron in combination with cystine The oxidation is inhibited by pyrophosphate M-30 and also by *aa* dipyridyl

It is thus apparent that though the oxidative process is not enzymic, the reduction process is influenced by a system part of which is undoubtedly enzymic, and we may assume that one or more dehydrogenase system is involved in the phenomenon A study of the effect of narcotics is therefore specially interesting It is well known that narcotics act in virtue of their capillary effects on the surface of catalysts, and it is thus reasonable to assume that a part or whole of the reducing process in liver may be due to a surface action It is also well known that the action of some of the dehydrogenases (Sen *loc cit*) which act in combination with cytochrome is inhibited by narcotics, and hence it is to be considered whether the inhibiting effect of urethane, as found in this work, is on a dehydrogenase system or not It is to be remembered that the effect of the urethane might be on the thermostable tissue and not on the thermolabile system, but a definite conclusion on the latter point cannot be reached until all the dehydrogenases have been isolated and the action of urethane on them is studied It is difficult to decide at present as to the substrates on which urethane is acting in a complicated system like liver-tissue The work described in this paper has not therefore cleared the outstanding

difficulties of the subject, but in addition to confirming some of the previous findings, a few facts which may be of interest in elucidating the mechanism of the reactions involved have been given It would therefore be well to *summarize* our knowledge of the present position of the subject —

Liver contains a reducing mechanism by virtue of which oxidized glutathione can be reduced and part of which is certainly enzymic in nature Ordinarily, fresh well-fed rabbit liver contains most of the glutathione in its reduced form (*cf* Hopkins and Elliot, 1931), and if the liver is allowed to stand under aerobic conditions, the SH remains at first constant for some time, but finally disappears, the oxidation process getting the upperhand When, however, liver-tissue, part of whose SH has been oxidized, is put under anaerobic conditions, its SH content begins to rise again, thus showing that the reducing system is still active It is apparent therefore that during the aerobic incubation of chopped liver, the velocity of reduction of the disulphide form (SS) may be equal to the velocity of oxidation of SH during the initial period in the case of well-fed rabbits, though ultimately the rate of oxidation predominates over that of the other Since the effect of this reducing system is to counteract the oxidizing effect of molecular oxygen it is of interest to inquire why the reducing mechanism fails after a short time, and it seems possible that the limiting factor in this case may well be the exhaustion of a hydrogen donator Obviously succinate, lactate or glucose have not served as hydrogen donators under the conditions of the experiments described in this paper, though glucose dehydrogenase system has been found in *in vitro* experiments to effect some reduction of oxidized glutathione, and this probably forms a part of the thermolabile reducing system of liver

It has been shown that the addition of extra disulphide (SS) to liver-tissue increases the amount of SH for some time This means that there was probably a lack of SS to be reduced by the reducing system which was not saturated by the already existing amount of SS Also in presence of excess of SS, the equilibrium $SS \rightleftharpoons SH$ may be shifted to the right during the initial period

The effect of urethane shows that at least a part of the reduction process is a surface reaction which is inhibited by narcotics as usual, but it cannot be definitely stated at present whether this effect is on a dehydrogenase system or not

The oxidation catalyst in liver seems not to be an enzymic one, but is a metal in combination with cystine It appears that Keilin's cytochrome-oxidase system does not take any part in the reversible oxidation-reduction of glutathione in liver It may be that we are dealing here with a new and highly specific oxidation catalyst

Finally, a few remarks may be made on the condition of glutathione in other cells It has been found by Meldrum (1930) that reduced glutathione in yeast cells is not appreciably oxidized by continued aeration and that this stability of the

thiol form is not due to any reducing mechanism as found in the case of liver-tissue This means that glutathione does not take any part in the oxidation-reduction phenomenon of yeast cell respiration and therefore the function of glutathione in yeast is quite different from that in mammalian tissue Another interesting fact observed by Meldrum (1932) is that in the reduction of SS in mammalian red blood cells, intact cellular structure is necessary, whereas from Mann's work it is clear that a cell-free system which will reduce oxidized glutathione, can be obtained from liver Hopkins and Elliot have also found that grinding the liver-tissue with sand does not inactivate, though it reduces to a certain extent, the reducing mechanism of the tissue so far as disulphide glutathione is concerned These facts thus indicate important differences between different cellular structures and seem to show that glutathione may not have the same function in different tissues

ACKNOWLEDGMENTS

The experiments given in this paper were carried out at the Biochemical Laboratory, Cambridge, and I wish to express my gratitude to Sir F Gowland Hopkins for the facilities given My thanks are also due to Mr F Ware, F R C V S , I V S , Director of this Institute, for his kind interest in this paper

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COMPLEMENT-FIXATION IN VARIOLA

BY

K V VENKATARAMAN, M D , D T M

(*From the King Institute, Guindy*)

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COMPLEMENT-FIXING anti-bodies in the serum of vaccinated calves were first demonstrated by Jobling (1906) Since then numerous observations have been published on the complement-fixation reaction with the serum of rabbit, calf and man infected with vaccinia using as antigen either vaccine lymph or crusts or pustules of smallpox Reports on human serum from cases of smallpox have been relatively few and conflicting in their conclusions

Gordon (1925) from a review of the literature and the result of his own work concluded that specific complement-fixing anti-bodies are of constant appearance in response to infection either by vaccinia or variola He himself obtained satisfactory fixation between anti-vaccinia serum and antigens of calf-lymph and smallpox crusts Schultz, Bullock, and Lawrence (1928) have criticized the positive reports of previous investigators as not demonstrating conclusively the specificity of the observed reactions on the ground that the virus preparations used had universally consisted of material contaminated with bacteria and adequate controls to eliminate this source of error had not been included They were themselves unable to detect any specific complement-fixation with the sera of rabbits immunized against bacteria-free brain-virus Bedson and Bland (1929) reported positive complement-fixation in the sera of 4 guinea-pigs immunized against a guinea-pig strain of vaccinia They included among their controls bacterial antigens prepared directly from the contaminating organisms Gilmore (1931), using a culture-virus prepared after the method of Maitland and Laeng (1930) as antigen, demonstrated fixation of complement in the sera of immunized rabbits and from 8 cases of smallpox He found that calf-lymph was an equally satisfactory antigen in the test and quite specific Thompson, Hazen, and Buchbinder (1932) have confirmed the specificity of the reaction in rabbits in an adequately controlled series of experiments Parker and Muckenfus (1932), using as antigen the contents of a pustule of

smallpox, obtained positive complement-fixation in the sera of each of 7 vaccinated persons and 14 out of 16 smallpox cases Ten control sera from cases of varicella, impetigo, pemphigus, etc , were negative

During the course of an investigation on the therapeutic value of convalescent sera in smallpox, undertaken by other members of the Institute, the opportunity of having a number of sera from known cases of smallpox was availed of to demonstrate the presence of specific complement-fixing anti-bodies in them

In the experiments here reported sera from 20 cases of smallpox, from 2 healthy adults who had had smallpox in childhood, 8 cases of chickenpox and 6 Wassermann positive sera were tested against an antigen of calf-lymph

Technique

The usual modified Wassermann technique was adopted and except for the following details does not require description *Antigen* Calf-lymph issued from the Institute for vaccination, which was a 1 in 5 glycerine dilution of the virus material, was diluted in 40 volumes of normal saline at the time of the test and the coarser particles allowed to settle down The slightly turbid supernatant was used as the antigen Preliminary tests had shown that it was not anti-complementary in double this strength *Sera* All the sera used in the test were previously inactivated in a water-bath at 56°C for half an hour They were used in dilutions ranging from 1 in 5 to 1 in 40 and the serum control in a dilution of 1 in 5 *Complement* Pooled guinea-pig serum which had been left in contact with the clot overnight This was titrated against 3 per cent sheep cells sensitized previously with 5 M H D of amboceptor Three M H D of complement was used in the tests throughout *Hæmolytic system* three per cent washed sheep erythrocytes sensitized with 5 M H D of amboceptor were used

The usual serum and antigen controls were included

In the tests, 0 1 c c was the unit of volume adopted One volume each of the serum dilution, antigen and complement were mixed and allowed to fix for 20 to 22 hours in a Copeland refrigerator (temperature 6°C to 8°C) Then one volume of sensitized cells was added and the tubes incubated in a water-bath at 37°C for half an hour The readings were taken immediately

Results

From the results recorded in the Protocol appended it will be seen that the sera from the cases of smallpox have fixed complement in dilutions up to 1 in 40 Only in 3 it has been as low as 1 in 10 There was no fixation of complement in the healthy adults who had smallpox several years before Number 20 in the Protocol had chickenpox and his blood was taken on the 11th day of the disease as one of the control series When the test was found to be positive, an inquiry into the previous history was made and it was found that he had been in hospital

only a month previously for modified smallpox The other chickenpox cases had not had smallpox and had not been vaccinated recently Wassermann positive sera were included in the controls to avoid the fallacy of non-specific fixation Wassermann tests were done on all the sera and the result is given in the last column of the Protocol —

PROTOCOL

Number	Disease	Day of disease	Serum dilution				Serum control, 1 in 5	Wassermann
			1 in 5	1 in 10	1 in 20	1 in 40		
1	Smallpox	35	++	++	+	±	−	−
2	„	44	++	++	++	+	−	−
3	„	36	++	++	++	+	−	−
4	„	24	+	+	−	−	−	−
5	„	24	++	+	+	±	−	−
6	„		+	±	±	−	−	−
7	„	34	++	++	++	++	−	−
8	„	21	++	++	++	++	−	−
9	„	16	++	++	++	++	−	−
10	„	26	++	++	++	++	−	−
11	„	15	++	++	++	++	−	−
12	„	26	++	++	++	++	−	−
13	„	20	++	++	++	++	−	−
14	„	29	++	++	++	++	−	D
15	„	26	++	++	++	++	−	−
16	„	37	++	++	++	++	−	−
17	„	25	++	++	++	++	−	−
18	„	15	++	++	++	++	−	−
19	„	30	+	±	±	−	−	−
20	„		++	?−	++	+	−	D
21	Chickenpox	11	−	−	−	−	−	−
22	„	20	−	−	−	−	−	Not done
23	„	16	?±	−	−	−	−	−

PROTOCOL—*concld*

Number	Disease	Day of disease	Serum dilution				Serum control, 1 in 5	Wassermann
			1 in 5	1 in 10	1 in 20	1 in 40		
24	Chickenpox	9	—	—	—	—	—	D
25	Smallpox several years before		—	—	—	—	—	—
26	"		—	—	—	—	—	—
27	Wassermann positive sera		—	—	—	—	—	++
28			—	—	—	—	—	++
29			—	—	—	—	—	++
30			—	—	—	—	—	++
31			—	—	—	—	—	++
32			—	—	—	—	—	++

— Complete hæmolysis ++ Complete inhibition of hæmolysis +, ± Partial inhibition of hæmolysis D doubtful

CONCLUSION

Specific complement-fixing anti-bodies are regularly present in the cases of smallpox and can be satisfactorily demonstrated by using a calf-lymph antigen

My thanks are due to Lieut.-Colonel H H King, Director, King Institute, Guindy, for permission to publish these results

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Ind Jour Med Res, XX, 4, April, 1933

THE SEASONAL PREVALENCE OF RATS AND RAT-FLEAS IN PARTS OF SOUTH INDIA

BY

LIEUT-COLONEL H H KING, I M S,

AND

P V SEETHARAMA IYER, M A

(From the King Institute, Guindy)

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INTRODUCTION

IN the course of a rat-flea survey of the Madras Presidency supervised by King and Pandit (1931), it was noticed that places surveyed between September and January showed higher flea indices than those which were surveyed during the hot and dry months of the year The data for this observation were obtained during a rapid general survey lasting for about 2 years of a number of different selected representative localities in the Presidency The ideal data for a study of the seasonal and climatic changes would be those obtained from localities in places representative of different types of climate during surveys carried out either continuously throughout the year or at least once in each different season Accordingly, it was decided to survey Saidapet, Bellary and Coimbatore on these lines Unfortunately, owing to financial stringency, the number of visits had to be curtailed The surveys were all done by the junior author (P V S I)

DESCRIPTION OF TOWNS SURVEYED

Saidapet, a low-lying town in the Chingleput district, is five miles from Madras and, from the point of view of a flea survey, may be taken to represent Madras City It has no record of any outbreak of indigenous plague and the species of flea present on the rats is *X astia* only May and June are the hottest months (mean temperature 86 5°F) The hot winds that blow during this period make the heat disagreeable This is mitigated by the south-west monsoon from July to September, but the effect is not great and the temperature does not come down much until the

breaking of the north-east monsoon in the latter half of October The mean 8 A M temperature for the whole year is 83°F , the temperature varies from 76°F in December and January to 110°F in June

Bellary is the headquarters of the Bellary district on the Mysore plateau and is 1,484 feet above sea-level The town is on a flat, almost treeless, expanse of black cotton soil with rocky hills here and there Bellary district has had plague almost every year Plague seems to be endemic in this district, the neighbouring parts of Mysore and the Bombay Presidency The species of rat-fleas present are *X astia* and *X cheopis*

The most marked feature of the Bellary climate (Table I) is the marked diurnal variation in temperature Days burning hot at noon are usually followed by cool nights From March to June the temperature is unpleasantly high It is highest in April—mean temperature 90 4°F This is followed by the south-west monsoon and then by a good cold weather from November to February when the temperature

TABLE I

Meteorological data for months of survey

Period of survey	Temperature					Humidity			Rain fall (Inches)
	Maximum		Minimum		Mean range	Mean dry bulb temp	Mean wet bulb temp	Mean humidity	
	Mean	Highest	Mean	Lowest					
Coimbatore									
December	83 5	89	67 5	61	16 0	72 7	68 6	81	4 93
April	95 8	99	75 0	72	20 8	81 7	74 8	71	1 84
July	86 0	90	71 4	70	14 6	75 5	71 4	82	1 03
Bellary									
May	102 1	107	78 5	70	23 6	73 5	64 7	58	0 81
November	85 5	90	67 9	62	17 6	73 5	69 2	79	1 02
Saidapet (Madras)									
December	83 0	85	70 6	64	12 4	75 8	72 5	84	4 76
March	89 6	93	72 6	69	17 0	80 4	75 2	78	
August	95 0	99	78 5	75	16 5	83 8	76 5	70	3 91

sometimes falls below 55°F The dryness of the air at Bellary makes the high temperatures far more bearable than in the damp coastal towns The driest and the dampest periods of the year are the end of March and the beginning of October respectively, the percentage humidity at these times was 43 and 88 in 1930

Coimbatore is the headquarters of the Coimbatore district which forms a buffer between the west coast and the eastern districts of the Presidency It is situated just to the east of the Palghat gap—a large break in the line of the Western Ghats—and lies south of the Mysore plateau It is 1,348 feet above sea-level Epidemics of plague used to recur every year until 1923 since when there has been no plague All *three* species of *Xenopsylla* are found The mean annual temperature is 77 6°F The month of April records the highest monthly average (85 4°F) The south-west monsoon which blows during the summer from mid-June to September, though it does not help Coimbatore by abundant rains, gives light showers and lowers the temperature September records a second rise in temperature (80°F) which again drops with the onset of the north-east monsoon in October giving a 'cold' weather from November to February The humidity is *generally* high It is highest in October, November and December, the mean figures being 83, 91 and 85 The minimum humidity is in May, the mean percentage being 77 The rainfall is comparatively small—average 27 inches—although situated in the path of both monsoons it does not fully benefit by either

Method of survey

Originally it was decided to survey the three places selected during (1) the cold weather after the north-east monsoon, (2) the hot weather, and (3) the south-west monsoon Coimbatore and Saidapet were surveyed three times covering the different seasons but unfortunately only two visits to Bellary were possible, the first during the hot months of April and May and the second early in November at the end of the south-west monsoon

The survey of each place during the different seasons extended to about 10 days on each occasion A general survey of the whole town was not done for the purpose of this inquiry—they had been fully surveyed in the general survey previously referred to (King *et al*, 1929, Pandit *et al*, 1930, King *et al*, 1931) The main bazaar and the residential area as represented by two or three streets away from the bazaar were selected for study At Coimbatore and Bellary, both centres of the cotton trade, separate data were obtained for fleas in cotton mills and cotton and gunny godowns The same localities were surveyed at successive seasons to get results of comparable value As far as possible the proportion of rats obtained from each area in the town was kept nearly uniform during the

different surveys All the surveys were carried out at a time when local rat epizootics or plague were non-existent

The usual technique was adopted Traps containing rats were enclosed in strong calico bags and so on

Rat density

Municipal rat-trapping has been in progress at Coimbatore and Bellary for a number of years On an average 22,380 rats are destroyed annually at Coimbatore while at Bellary the figure is nearly 27,650 per year The figures for the average number of traps distributed every month are not available The actual figures for the rats caught at Coimbatore in different seasons in the five years 1926 to 1931 are as follows —

Season and months	1926—1927	1927—1928	1928—1929	1929—1930	1930—1931	Average for 5 years for each season
Cold weather, November to February	6,262	7,185	6,882	8,370	9,232	7,586
Hot dry weather, March to June	6,946	6,184	6,116	7,680	8,860	7,157
Hot weather with monsoon, July to October	7,447	6,745	6,305	9,582	7,675	7,551

If we assume that the number of traps set was roughly the same for each month, then the figures for the rats caught each month in the five years 1926 to 1931 show that the density of rats is *practically uniform* except for a slight drop in the hotter and drier months of the year It is interesting to speculate whether this last is an effect of the hot months on the rats themselves or on their catchers—for the effect of hot weather on human activities must not be lost sight of in work such as this No deductions can be made from the figures for rats caught in Bellary as they have been kept for only one year

The density for *Rattus rattus* at Coimbatore is the lowest among the areas surveyed On the other hand mice were relatively abundant Not a single mouse was obtained at Bellary or Saidapet If we take the number of rats killed as an index of the number of rats living, we find that the trapping of rats in the last 5 years at Coimbatore has not resulted in any appreciable decline in the rat population—there has even been a slight increase As regards our own survey, the figures for rat density are given in Table II They are best considered separately for each area

TABLE II

Rats

Period of survey	NUMBER OF *R rattus* CAUGHT			*R rattus* DENSITY (*R rattus* FOR 100 TRAPS)		
	Whole area	Bazaar	Residential area	Whole area	Bazaar	Residential area
COIMBATORE						
13-12-1930—24-12-1930	142	53	56	28 8	29 3	26 4
23-3-1931—8-4-1931	123	51	59	22 3	27 4	22 4
16-7-1931—26-7-1931	199	74	85	30 9	38 9	27 8
BELLARY						
28-4-1931—7-5-1931	235	79	77	60 1	87 8	47 0
30-10-1931—7-11-1931	179	58	57	47 2	54 7	30 2
SAIDAPET						
1-12-1930—12-12-1930	142	80	62	60 0	108 0	38 0
11-3-1931—22-3-1931	157	96	61	64 0	79 0	49 0
2-8-1931—22-8-1931	114	58	56	41 5	40 0	43 0

Coimbatore —Density least in both bazaar and residential areas in the second survey which was at a hotter period than the other two (*see* Table I)

Bellary —No comparison for rat densities is possible because municipal trapping in the area surveyed did not precede the first survey, it did precede the second and lowered the figure for rats

Saidapet —The density was least in the third survey which, as seen from Table I, was at the hottest period so far as these surveys are concerned

PREGNANCY AND REPLENISHMENT RATES

The available figures are given in Table III Unfortunately data were not kept for some of the surveys so that the comparison between cold and hot weather is only possible for Bellary Here there is a slight increase in the size of the litter in the cold weather

TABLE III

Average number of fœtuses in pregnant females

Period of survey	Coimbatore			Period of survey	Bellary			Period of survey	Saidapet		
	Whole area	Bazaar	Residential area		Whole area	Bazaar	Residential area		Whole area	Bazaar	Residential area
April	5 8	6 0	5 0	November	4 92	4 57	4 75	March	4 97	5 77	4 31
July	5 0	6 8	4 0	May	4 37	4 25	4 55	August	4 8	4 8	4 9

Fleas.

General flea index for Rattus rattus (Table IV and the Chart)

It is fortunate that no epizootic of plague was prevalent at any of the places surveyed This disturbing factor therefore cannot vitiate our conclusions At successive surveys, care was taken to catch roughly the same proportion of rats in the localities surveyed

(*a*) The most outstanding feature in all the places surveyed was the markedly high flea indices for the cold weather (December) These cold weather high indices were noticed both in bazaars and residential areas in all the three places The December flea indices for cotton mills at Coimbatore (14 1) and cotton godowns at Bellary (13 14) were the highest recorded during the survey The general flea index for bazaar areas in Saidapet and Coimbatore during the same period was higher than for residential areas Walker *et al* (1931) working in Hyderabad (Deccan) also found that the flea index was very high during the months of September to December when the optimum conditions for flea breeding, such as a low temperature and a high percentage humidity, were present

(*b*) The surveys at Coimbatore and Bellary in the hot weather show a marked diminution in the number of fleas The lowest indices were obtained in April and May This reduction was very much greater in residential areas, cotton mills and godowns than in bazaars The rapid decline in the incidence of human plague at the beginning of the hot weather must surely be related mainly to this reduction in the number of fleas The survey of Saidapet which was done in the middle of March when hot weather conditions had not yet established themselves, as is to be

TABLE IV

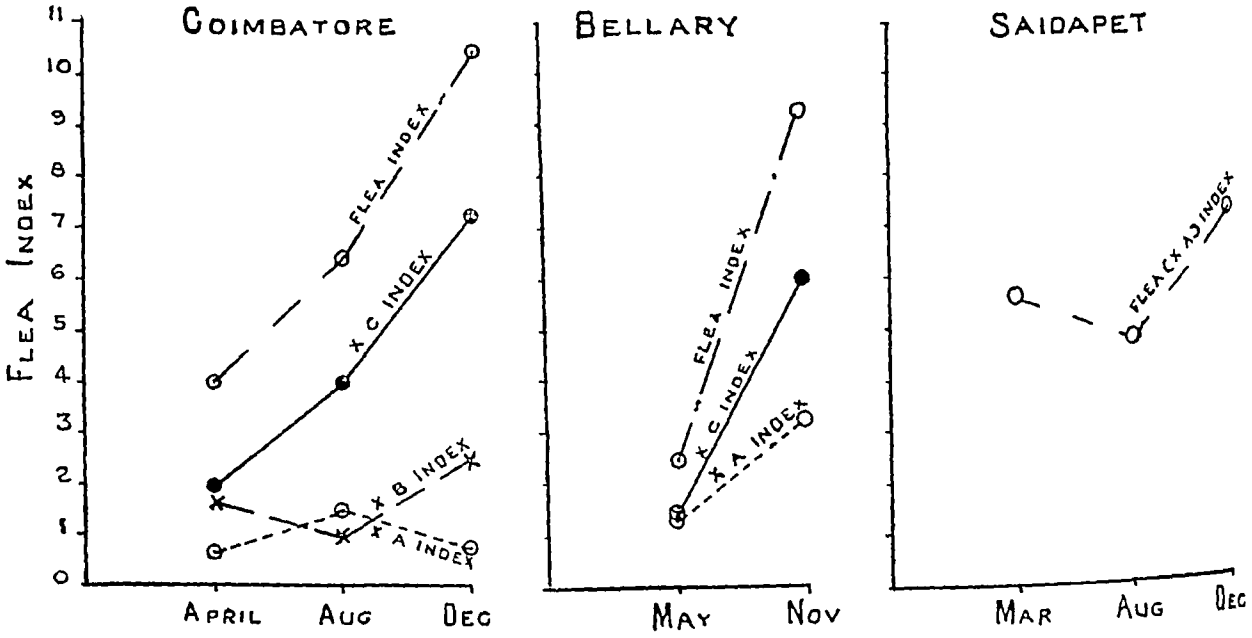
Flea indices

Period of survey	COIMBATORE				Period of survey	BELLARY				Period of survey	SALEM		
	Whole area	Bazaar	Residential area	Cotton mills		Whole area	Bazaar	Residential area	Cotton and gunny godowns		Whole area	Bazaar	Residential area
General flea index for different seasons													
December	10 4	10 8	7 7	14 1	November	9 25	6 16	8 04	13 14	December	7 3	8 1	6 3
April	4 0	4 3	4 0	3 3	May	2 52	3 08	2 10	2 38	March	5 6	6 34	4 42
July	6 43	5 64	7 85	4 93						August	4 73	5 79	3 63
X astia *index for different seasons*													
December	0 7	1 3	0 45	0 2	November	3 24	1 34	1 93	6 12	December	7 3	8 1	6 3
April	0 6	0 92	0 46	*Nil*	May	1 25	1 32	0 91	1 51	March	5 6	6 34	4 42
July	1 46	1 85	1 67	0 28						August	4 73	5 79	3 63
X cheopis *index for different seasons*													
December	7 2	6 4	4 9	12 2	November	6 01	4 82	6 11	7 02				
April	1 9	2 0	1 5	3 3	May	1 28	1 76	1 19	0 87				
July	3 91	2 73	4 67	4 48									
X braziliensis *index for different seasons*													
December	2 5	3 1	2 4	1 7									
April	1 6	1 4	2 0	*Nil*									
July	1 07	1 05	1 51	0 17									

expected, did not show the same degree of reduction either in the general flea index, or in the flea index for its only species, *X astia*, as Bellary and Coimbatore which were surveyed in April and early May, but as was reported for rat density, the weather was hottest during the third or August survey in Saidapet

(c) The marked contrast between Saidapet and Coimbatore as regards climate in this month of August is reflected in the figures for the flea index during that month Whereas Coimbatore after the onset of the south-west monsoon on the west coast gets considerably cooled, Saidapet has a continuous record of high

CHART



temperature In Saidapet (Madras) the hot weather conditions persist for a period of nearly seven months from April The flea index for this month (August) in Saidapet showed a further decline over the figures for March, while at Coimbatore there was a distinct tendency for the average number of fleas per rat to increase as a result of the influence of the south-west monsoon which considerably cooled the temperature The influence of the long hot weather in Saidapet and of the monsoon in Coimbatore on flea prevalence is perceptible in the figures obtained separately for bazaars, residential areas and cotton mills The absence of species of *Xenopsylla* other than *X astia* in Saidapet and the great preponderance of this species in other places in the east coast may possibly be due to a greater ability to survive long spells of hot weather

A rise of temperature can affect flea prevalence in various ways —

1 High temperature and low humidity or both may shorten the life of fleas

2 With a rise of temperature there is usually a concomitant reduction of flea activity (Hirst, 1924–27) and so a smaller number will be found on each rat leading only to an apparent and not a real reduction It is possible that fleas tend to lead a resting life during the hot weather, feeding seldom or not at all

INFLUENCE OF SEASON ON ASSOCIATION OF RATS WITH FLEAS (Table V)

An attempt was made to estimate the number of rats from which no fleas were obtained during the different seasons of the year The difficulty of getting separate figures for individuals when more than one rat was caught in a trap necessitated apportioning the fleas gathered from a trap equally between the rats caught in that trap Despite this adverse factor, the figures for rats without fleas (Table V) yield results of some importance The percentage of rats *without* fleas on them

TABLE V

Association of fleas with rats at different seasons (whole area)

Period of survey	PERCENTAGE OF *R rattus* WITHOUT FLEAS			PERCENTAGE OF *R rattus* WITHOUT *X astia*			PERCENTAGE OF *R rattus* WITHOUT *X braziliensis*			PERCENTAGE OF *R rattus* WITHOUT *X cheopis*		
	Coimbatore	Bellary	Saidapet	Coimbatore	Bellary	Saidapet	Coimbatore	Bellary	Saidapet	Coimbatore	Bellary	Saidapet
November		*Nil*			12 3						3 4	
December	1 41		0 7	64 1		0 7	34 51			1 41		
March			1 91			1 91						
April	5 7			60 98			32 52			21 14		
May		5 53			26 8						27 2	
July	1 01			43 72			50 75			2 02		
August			2 63			2 63						

showed a definite rise during the hot weather This influence was particularly marked in the case of *X cheopis*, for which the figures run from only 1 4 per cent and 3 4 per cent in Coimbatore and Bellary respectively for the cold weather to 21 1 per cent and 27 2 per cent in the hot weather It is important to remember that when we are discussing the effect of season on fleas in surveys such as this, we are really discussing variations in the total number found *on rats* which is

not necessarily the same thing as the total number existing, for, as stated above, apart from a diminution in numbers, hot weather may cause a diminution in the *activity* of fleas As regards the transmission of plague, it is this activity of fleas and the number of fleas found on rats with which we are directly concerned, so this great rise in the hot weather in the percentage of rats found without any *cheopis* is very suggestive of how seasonal factors act in stopping plague

X astia (Table IV and Chart)

This flea was present in all the three areas surveyed The specific *X astia* index follows the general flea index in its variations

In Saidapet, since *X astia* is the only species present, its index is that of the general flea index whose movements have already been discussed

In Coimbatore, where all the three species of *Xenopsylla* are present on the rats, the *astia* index was very low in the month of April With the increase of humidity and the fall of temperature occurring in August the *astia* index showed a marked rise In the month of December, when cold weather conditions had set in and the temperature considerably lowered, the index for this flea showed a tendency to decrease This was probably mainly due to other species of *Xenopsylla* finding the temperature conditions in December more suitable for development than *X astia* We suggest that the increase in the other species of *Xenopsylla* particularly *X cheopis* tended to crowd out *X astia* and reduce its index for this period The same feature was seen when the figures for the bazaars and mills were examined The index for the residential area at Coimbatore was fairly steady during the cold and early hot weather but the conspicuous south-west monsoon rise was evident—the third survey

Bellary was surveyed in November when the south-west monsoon had abated and the north-east monsoon was just commencing The *astia* indices were then considerably higher in the different areas than the figures obtained during May This was conspicuous in the cotton godowns where the number of *X astia* per rat in November was four times higher

X cheopis (Table IV and Chart)

This is the predominant flea in Bellary and Coimbatore Observations indicate that the average number of *X cheopis* per rat was the lowest in April and May The index for this species at Coimbatore gradually rose as the temperature fell during the south-west monsoon and the index reached its peak in December concomitant with the very low temperature prevailing during this period The indices of both *X astia* and *X cheopis* show a tendency to rise as we pass from the summer conditions prevailing in April and May to the monsoon conditions in August with *X astia* taking the lead The higher humidity with the lower temperature in August resulted in a marked increase of the *astia* population But the *still lower*

temperature prevailing in December seemed particularly favourable to X cheopis, for the index for this flea rose considerably during this period and as suggested, in consequence, kept down the *astia* population found on rats The marked disparity between the indices in the cool and hot months of the year showed definitely that this species was more susceptible to changes of temperature than *X astia* and also that a continued low temperature was more favourable to *cheopis*

It has been suggested that environmental factors influence greatly the flea index and relative prevalence of species In bazaars for instance some may consider that the flea population is subject to fluctuations due to influx of merchandise But if this factor, which may vitiate the results to some extent, exists to any appreciable extent it is surprising that the figures obtained for bazaars bear out every one of the conclusions based on residential areas No marked discrepancies as between residential areas and bazaars were noticed at any of the places surveyed

Another interesting feature noticed was the abnormally high proportion of *X cheopis* found in cotton mills at Coimbatore The percentage of *X cheopis* varied from 87 to 100 as compared with a variation from 47 to 69 for the whole town The *cheopis* index in the mills for the month of December was also very high (12 2) Cotton not only seems to afford optimum conditions for rat and flea breeding, but the conditions under which it is kept in mills—very often with artificial raising of humidity—also seem to particularly favour *X cheopis*

X braziliensis (Table IV and Chart)

In this survey this flea was present only on rats from Coimbatore The chief habitat of this flea is the Mysore plateau and it is surprising that Bellary which lies on a lower plateau to the north of Mysore should be free It has, however, been recorded in Harpanahalli, a small town in Bellary district lying in close proximity to the Mysore borders

The variations in the *braziliensis* index were not so well marked as for *cheopis* The specific index was not very high at any one period The general rise in total flea index during December was also noticed here The indices for the whole area were 1 6, 1 07, and 2 5 for the April, July and December surveys respectively Whereas both *X astia* and *X cheopis* show signs of higher indices during the monsoon months of July and August with *cheopis* reaching the peak in the month of December at the expense of *X astia*, the *X braziliensis* index shows a definite decline in July and shows the first signs of increase only with the onset of the cold weather This suggests that this flea requires a still lower temperature than *cheopis* and that this is probably why in South India its distribution is mainly confined to the Mysore plateau

RELATIVE PROPORTIONS OF THE THREE SPECIES OF *Xenopsylla* DURING DIFFERENT SEASONS (Table VI)

The figures for the specific flea indices suggest that the influence of temperature and humidity on the three species of *Xenopsylla* follows nearly the same lines as for the general flea index This is true when we consider the specific index of each species without reference to the other species of *Xenopsylla* in the locality, but when the *relative proportions* of the three species on rats during different seasons are taken into consideration, it is noticed that climatic variations do not affect the prevalence of the three different species of *Xenopsylla* to the same extent and degree *The three species differ markedly in their capacity to adapt themselves to their environmental climatic* conditions

TABLE VI

Relative proportions of the three species of Xenopsylla *on rats at different seasons*

Period of survey	WHOLE AREA			BAZAAR			RESIDENTIAL AREA		
	X astia	*X cheopis*	*X braziliensis*	*X astia*	*X cheopis*	*X braziliensis*	*X astia*	*X cheopis*	*X braziliensis*
				COIMBATORE					
December	7 0	69 0	24 0	12 4	58 8	28 7	5 8	63 7	30 5
April	14 9	46 8	38 3	21 5	46 1	32 4	11 4	37 8	50 8
May	22 6	60 7	16 6	32 9	48 4	18 7	21 3	59 5	19 2
				BELLARY					
November	35 02	64 98		21 85	78 15		24 02	75 98	
May	49 4	50 6		42 8	57 2		43 2	56 8	

The figures for the proportion of *X astia* to total fleas during different seasons confirm the previous conclusion that this species commences to increase in numbers

when we pass from the higher temperatures and lower humidity prevailing in the summer months to the monsoon months with their lower temperatures and higher humidity The increase is more marked in *X astia* than for the other species of *Xenopsylla*

On the onset of the cold weather with its considerably lowered temperature, *X cheopis* finds its optimum conditions and increases considerably in numbers, so much so that nearly three-fourths of the fleas from the residential area belong to this species This remarkable increase of *cheopis* naturally affects the proportion of *X astia* on rats which becomes very low in December This shows clearly that *cheopis* is better adapted to thrive during the cold months of the year

The *relative* proportion of *X braziliensis* was highest in the April survey at Combatore—the highest absolute number was in December This, we suggest, is probably due to the period of most active multiplication of this species being later than the other two, i e , that it does not begin to multiply until the cold weather has well set in This later multiplication may be the reason why the early hot weather shows a relatively smaller diminution of this species in April than the others As seen from the specific index there is a real absolute diminution at this time Possibly this species, though prefering a colder climate than the other two, may also prefer a drier climate, and so will not respond to the south-west monsoon like the other two species If so, it will also be *less* damaged by the increasing dry heat of the early hot weather than the other two species

The comparatively higher indices for *X astia*, which is the only flea on Saidapet rats during the hot weather months from March to September, also serve to confirm the inference that *X astia* is able to survive a long spell of dry and hot weather

The tentative conclusions that may be drawn from these rather scanty observations are that—

1 Flea life is at its lowest during the hottest months

2 With the onset of the south-west monsoon, with cooler and more humid weather, *astia* and *cheopis* begin to multiply

3 This multiplication for *cheopis* is at its highest in the cold weather after the north-east monsoon in November

4 *Cheopis* shows by far the greatest seasonal changes—this is well brought out by the Chart *Astia* appears to be a hardier species less susceptible to the influence of climate

5 *Braziliensis* does not increase until cold weather conditions have been reached

Sex proportions (Table VII)

A higher proportion of males in *X cheopis* and *X braziliensis* and of females in *X astia* was seen in all the places surveyed

There was no marked association of sex prevalence with season *X braziliensis* in Coimbatore alone showed a higher percentage of males during the early hot weather of April

TABLE VII

Effect of season on sex proportions (whole area).

Period of survey	Percentage of male fleas to total fleas			Percentage of male *X astia* to total *X astia*			Percentage of male *X braziliensis* to total *X braziliensis*			Percentage of male *X cheopis* to total *X cheopis*		
	Coimbatore	Bellary	Saidapet	Coimbatore	Bellary	Saidapet	Coimbatore	Bellary	Saidapet	Coimbatore	Bellary	Saidapet
November		59 2			39 7						69 7	
December	59 9		50 8	42 2		50 8	63 4			60 9		
March			54 4			54 4						
April	62 4			40 5			74 3			55 4		
May		45 7			33 8						57 3	
July	51 2			36 2			54 0			56 0		
August			45 6			45 6						

SUMMARY

So far as our rather scanty data go we may say that the following conclusions appear to be justified and may perhaps hold for South India generally —

Rats

1 The density of rats as judged by the number of rats caught for 100 traps was higher during the cold weather (November to February) The density decreased during the hot weather followed by a south-west monsoon rise in Coimbatore As hot weather conditions continue at Saidapet during August the monsoon rise was not perceptible

2 Municipal trapping for several years at Coimbatore has not resulted in any appreciable decline in the rat population

3 The average number of young ones per litter remained fairly uniform for any one place at all seasons of the year

4 Seasonal changes had very little effect on the pregnancy and replenishment rates A slight cold weather rise in the proportion of females and pregnant females was noticed at Bellary

Fleas

1 The most marked feature was the low flea indices—both general and specific—during the hot weather months when the temperature was high and the humidity comparatively low

2 *X astia* appears to react first to the lowering of temperature and increase of humidity which occur during the south-west monsoon months of July and August by increasing in numbers

3 With the onset of the cold weather after the north-east monsoon, *cheopis* continues to increase and increases very greatly At this time *braziliensis* begins to increase

4 *Cheopis* seems to be the species most affected by seasonal changes

5 The proportion of rats without fleas was higher in all three places during the months of April and May than during the cold months The percentage of rats without fleas in Saidapet increased in August while at Coimbatore there was a tendency to regain cold weather proportions

6 There was striking disproportion between the number of rats without *cheopis* in the cold and hot months of the year, an evidence of the extreme susceptibility of *X cheopis*

7 There was no marked association of sex prevalence with season

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A SURVEY OF THE FLORA AND FAUNA OF THE WATER-SUPPLIES OF THE MADRAS PRESIDENCY

BY

S A RAFAY M SC

INTRODUCTION

BY

LIEUT-COLONEL H H KING, I M S,
Director, The King Institute, Guindy

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INTRODUCTION

1 ONE of the commonest forms of water-supply in this Presidency is a shallow artificial lake Such water stored in the open for six months and more produces immense quantities of algæ This causes difficulties in subsequent purification processes due both to the presence of living algæ and to the addition of colloidal organic matter produced by their decay Accordingly it was considered advisable to learn as much as possible of the flora and fauna of the waters and, to this end, the Indian Research Fund Association were asked for a grant of Rs 6,360 to finance a survey This they very kindly provided Mr S A Rafay, M SC, who is a botanist and biochemist with previous experience of this kind of work, was engaged from April 1931 to July 1932 when the survey was completed

2 Several supplies were selected not just for their individual importance but also for their representative character, so that stored lowland surface, stored upland surface, stored river, direct river and well waters are represented Most were sampled at least twice so as to investigate both hot and cold weather conditions Since the Madras water-supply well exemplifies the difficulties of purifying a stored surface and stored river water, it was thoroughly sampled both in the lake and in the filter beds—11 sets of samples at different times

3 Much useful information of the actual species found, of their relative numbers, and of their seasonal changes was obtained A further benefit has been that the staff of the Institute have been assisted in their knowledge of this subject and of methods of cultivation of various species

4 In addition to the survey, experiments were conducted to ascertain first the length of life of the commoner species found and second to estimate the *amount of debris* each species produced on its death This point of the relative importance of species judged not by their relative numbers at any one moment, but by the relative amounts of organic matter they add to water is too often forgotten So far as I know these are the first experiments to determine this point Judged by these tests the most important forms in Madras are the blue green algæ *Scytonemæ*—the iron bacterium, *Cladothrix* and the higher alga *Chara*

5 Experiments on the action of copper sulphate were also done and the minimum lethal dose of copper was determined for many species This determination along with the work just mentioned was most useful, for we found that the most abundant species and those adding *most* to the organic matter in the Madras water-supply required but a small dose of copper—0 3 parts per million Accordingly I was able to advise the Madras Corporation that copper sulphate treatment of their chief lake would probably be successful The advice was accepted and in August 1932 ten tons of copper sulphate at a cost of Rs 3,500 was added to the lake—this gave a solution of 0 3 parts per million The subsequent clarification of the lake water and the reduction of organic matter have been striking In September the average reduction of organic matter was 22 per cent and this too at a time when usually the organic content is at its highest The chlorine dose required for the raw water fell from 2 parts per million in August to a minimum of 0 6 parts in September

[H H K]

SECTION I

Method of collection of total counts

There are two methods in use, of collecting and counting micro-organisms—one is the 'Plankton net' method and the other the 'Sedgwick-Rafter' method

In the 'Plankton net' method the loss of smaller organisms by leakage through the meshes of silk is very great and many of the delicate organisms are crushed on the net The method of estimating the volume and weight of the plankton is also inaccurate and the results cannot be depended upon within 50 per cent

Accordingly the other or Sedgwick-Rafter method was followed which consists of collecting the samples in a bottle, filtering a measured quantity of water from this sample (through a layer of sand upon which the organisms are retained),

separating the organisms from the sand by washing with distilled water and decanting, enumerating the organisms found therein, and then calculating from this the number of organisms in the sample of water examined For filtration sand that will pass through a sieve having 60 meshes to an inch but which will be retained by a sieve having 100 meshes to an inch was used

To ensure the taking of representative samples they were taken at various depths and places

SECTION II

Culture methods

The majority of algæ occur in mixtures in which one form is intermingled with another and the difficulty of separating them is enhanced when there are present one or more species of blue green algæ with their gelatinous investments No amount of shaking will separate them and they have to be placed in pure water when the most resistant outlasts others In addition to this different media have to be used which will promote the growth of specific organisms By allowing the medium to dry gradually gametes may be formed and these bodies may be transferred to suitable media For planktons consisting only of unicellular and colonial forms a small quantity of the material is shaken up with a large bulk of water and a drop of this transferred to the culture medium, if this does not give a pure culture the same operation is repeated till a pure culture is obtained

Thus to isolate pure forms several methods have to be used and the operation takes five weeks or more

A list of the media successfully used is given in Appendix I

SECTION III

Red Hills Lake

Cold weather —Samples collected on 25th November, 1931, and 6th January, 1932

On the sides of the filter basins *Scytonema** was the predominant type forming a layer about three-quarters of an inch thick It constituted about 90 per cent of the flora attached to the sides, the rest being *Nitzschia*, *Navicula* and *Staurastrum* Filaments of *Oscillaria** were also present On the border of the lake attached to the stone of the revetment (under water-level) were *Scytonema* and *Oscillaria* with *Nitzschia* and *Navicula* in small patches Colonies of *Nostoc* were also present in separate groups

* Organisms known to give trouble in water supplies by producing odours are asterisked here and elsewhere

The number of organisms per c c of water are given in Table I In these and other tables genera are arranged in the following order of groups (*A*) blue green algæ, (*B*) the chlorophyceæ or green algæ, (*C*) diatoms, (*D*) fungi, and (*E*) animal groups

TABLE I

Genera	25TH NOVEMBER, 1931			6TH JANUARY, 1932		
	LAKE WATER		Entrance to conduit	LAKE WATER		Entrance to conduit
	From surface	At 12′ depth		From surface	At 12′ depth	
A Lyngbya				27	44	
A Nostoc	1	2	5	2	4	
A Phormidium	2	2		31	60	31
B Chlorella	4		2	3		
B Staurastrum				12	11	6
B Tetraspora				8	4	5
B Ulothrix	6	1	4	63	58	14
C Amphora	110	100	37	121	118	32
C Fragilaria	10	8		30	29	
*C Melosira**	35	37	40	21	30	27
C Nitzschia	46	42	68	105	77	101
*C Synedra**	52	63	17	38	51	13
*D Cladothrix**	20	18	6	43	10	14
*D Crenothrix**				54	38	22
E Paramœcium	38	27	30	24	15	32

As usual the filamentous forms were confined to certain areas They were found in floating clusters But short filaments were also found that had been separated from the main clusters Such forms probably result from the breaking up of individual filaments As these broken filaments are capable of independent life they were counted as distinct individuals

Hot weather —3rd April, 1932 to 8th May, 1932

*Chara** and *Nais* appeared in the shallow portions of the lake On their stems and branches *Anabæna**, *Rivularia* and *Nostoc* were commonly found Attached

to the stones of the revetment were *Scytonema* and *Cladothrix* with numerous *Paramœcia*

On the sides of the filter basins *Scytonema* constituted three-fourths of the slimy carpet *Nitzschia*, *Navicula* and *Oscillaria* being associated with it

Counts per c c were as below —

TABLE II

Genera	3rd April, 1932			8th May, 1932		
	Lake water		Entrance to conduit	Lake water		Entrance to conduit
	From surface	At 12′ depth		From surface	At 12′ depth	
A *Microcystis**	110	123	90	110	196	96
B *Ankistrodesmus*				200	209	112
B *Cœlastrum**	5	7	1	10	12	
B *Euglena*	2	2	3	6	4	4
C *Asterionella**	102	32	122	58	15	87
C *Fragilaria*				113	161	75
C *Navicula*	234	289	145	416	337	228
C *Nitzschia*	439	307	219	789	525	542
C *Synedra*	236	286	201	139	163	115
D *Cladothrix*	57	62	84	57	62	84
D *Crenothrix*	40	22	52	173	226	230
E *Paramœcium*	25	27	30	40	42	53

Compared with the findings in Table I, the increase of several organisms, especially of *Nitzschia* and *Crenothrix*, and the appearance in large numbers of *Microcystis*, *Ankistrodesmus* and *Navicula* are noteworthy

South-west monsoon —19th July, 1931

*Chara**, in the Red Hills Lake, increased till it appeared to constitute about half of the entire flora of the lake *Nitella* and *Nais* were associated with *Chara* on the southern side of the small island facing the wind On the northern side of the same island where the water was comparatively calm, *Navicula*, *Anabœna* and *Synedra* were attached to *Chara* The lake also showed

a rich growth of the common grasses which on their decay harboured *Diatoms*, *Anabæna*, *Utricularia* (an angiosperm) *Crenothrix* and *Cladothrix* (fungi) In the lake water vascular bundles of angiosperms with a large number of *Paramœcia* were observed

The sides of the filter basins had growths of *Scytonema* associated with which were *Mougeotia*, *Amphora*, *Synedra*, *Navicula*, *Asterionella*, *Tribonema** and *Cladothrix*

TABLE III

Genera	Collection on 19th July, 1931		
	Lake water		Entrance to conduit
	From surface	At 12′ depth	
B Chlorella	305	195	215
B Ulothrix	400	212	338
C Amphora	415	380	324
C Asterionella	52	18	23
C Navicula	290	286	272
C Nitzschia	900	616	692
C Pinnularia	115	128	94
C Synedra	360	442	356
D Crenothrix and *Cladothrix*	4		3

These results refer to 1931 whereas those recorded for the previous season refer to 1932 If we may take the results as truly representing a seasonal change the main differences observed are —

1 The disappearance of *Microcystis*, *Ankistrodesmus* and the very great diminution in *Crenothrix* and *Cladothrix*

2 The increase in *Nitzschia, Synedra, Ulothrix* and *Pinnularia* and the growth of *Chara* on the sides in the shallow portion of the lake—as it is a fixed species it is not included in the tables which refer to species found in water-samples

North-east monsoon —16th October, 1931

Chara, in the Red Hills Lake, decreased as also the organisms associated with it

Scytonema was reduced to one-half of the flora in the carpet attached to the sides of the filter basins, the rest consisting of *Phormidium, Cladothrix, Beggiatoa*, Rivularia, Navicula* and *Spirogyra* *

TABLE IV

Count per c c of water collected on 16th October, 1931

Genera	LAKE WATER		Entrance to conduit
	From surface	At 12′ depth	
B *Chlorella*	415	208	385
B *Ulothrix*	440	112	316
C *Amphora*	355	217	291
C *Asterionella*	28	1	16
C *Navicula*	210	193	198
C *Nitzschia*	700	414	592
C *Pinnularia*	85	62	70
C *Synedra*	240	325	230
C *Tabellaria*	126	85	110
D *Cladothrix*	10	22	25
D *Crenothrix*	24	37	32

Compared with Table III there does not seem to be any marked change *Tabellaria* is a new genus

SUMMARY OF SEASONAL CHANGES

Scytonema was found throughout the year associated with *Oscillaria, Nitzschia* and *Navicula* in the cold and hot weather and with *Mougeotia* and *Tribonema* during the south-west monsoon and with *Spirogyra* and *Phormidium* during the north-east

monsoon *Chara* appeared in the middle of the hot weather, increased considerably during the south-west monsoon and disappeared by November The cold weather gave the greatest variety of organisms but the least in total number *Navicula*, *Nitzschia* and *Synedra* increased greatly in the hot weather, and *Amphora* and *Synedra* during the south-west monsoon and *Spirogyra* and *Phormidium* appeared during the north-east monsoon

Kilpauk filters—19th January, 1932, and 20th February, 1932

Cold weather—On the surface of the experimental percolating pre-filters PF_2 and PF_1 there was a slimy carpet of *Cladothrix*, *Crenothrix*, *Diatoms* and *Scytonema* Algæ, etc, were not found in the effluent of the submerged sand filter

TABLE V

Counts per c c of water collected on 19th January, 1932, and 20th February, 1932

Genera	19TH JANUARY, 1932					20TH FEBRUARY, 1932		
	Raw water	PF_4		PF_5		Raw water	PF_5	
		Sprayed water	Effluent	Sprayed water	Effluent		Sprayed water	Effluent.
A. Anabœna .						47	42	7
*A Clathrocystis**	21	16						
A Phormidium	120	136	126	134	12	.		
C Fragilaria	4	3	1	4	1			
C Navicula	110	89	26	52	27	471	480	218
C Nitzschia	291	200	184	187	92	518	484	108
C. Pinnularia	23	8				16	14	5
C Synedra	17	16	6	16	3	71	60	8
E Paramœcium	42	39	12	40	5	28	19	6
E Vorticella	24	20	4	18		16	5	2

Hot weather—30th April, 1932, and 27th May, 1932

Samples of water reaching the pre-filters at Kilpauk showed a large quantity of flocculent matter of fungoid origin In the decaying *flocs* were entangled *Amphora*, *Navicula*, *Pleurococcus*, *Nostoc*, *Ulothrix*, *Paramœcia* and *Rotifers* Annular vessels of angiosperms and filaments of *Beggiatoa* were also in evidence

TABLE VI

Counts of organisms per c c of water

Genera	27TH MAY, 1932			30TH APRIL, 1932		
	Raw water	PF_5		Raw water	PF_5	
		Sprayed water	Effluent		Sprayed water	Effluent
A *Anabœna*	128	121	109			
A *Microcystis*	75	66	96	16	9	2
A *Oscillaria*	141	120	167	48	51	64
A *Phormidium*	240	254	87	122	156	72
B *Mougeotia*	57	45				
C *Diatoma*	101	97	29	78	65	17
C *Navicula*	371	400	100	112	202	178
C *Nitzschia*	442	460	356	506	591	160
D *Cladothrix*	25	18		8	6	
D *Crenothrix*	60	56		51	60	2

Compared to Table I the increase in *Nitzschia*, *Navicula*, *Anabœna* and *Phormidium*, and the appearance of *Mougeotia* is noteworthy

South-west monsoon—15th August, 1931

The surface of the percolating pre-filter (No PF_4) had a slimy growth of *Cladothrix*, *Crenothrix* and *Scytonema* The same growth was noticed on the surface of pre-filter (PF_5) also but only in small patches On the drains the growth of *Mougeotia* and *Chætophora* was observed

TABLE VII

Counts of collection on 15th August, 1931

Genera	Raw water	Per c c of water			
		PF_4		PF_5	
		Influent	Effluent	Influent	Effluent
A Clathrocystis	35	18	24	12	9
A Nostoc	5	3	1		
A Phormidium	16	37	9	48	36
C Diatoms	139	140	83	160	78
E Paramæcium	15	11	1	10	4

Compared to Table VI, note the disappearance of *Nitzschia*, *Navicula*, *Diatoma* and *Oscillaria* and the decrease in number in general

North-east monsoon —Counts of collection on 28th October, 1931

TABLE VIII

Genera	Raw water	Per c c of water			
		PF_4		PF_5	
		Sprayed water *	Effluent	Sprayed water	Effluent
A Phormidium	17	17		30	5
C Melosira	4	5			
C Navicula	36	31	60	40	30
C Nitzschia	64	48	50	68	44
C Synedra	60	55	28	44	28

* Note the appearance of *Synedra*, *Nitzschia* and *Navicula* compared to Table VII

Kilpauk water showed a variety of organisms like Red Hills Lake water in cold weather, numerically, however, they were quite low *Amphora*, *Navicula* and *Nitzschia* increased considerably in hot weather There was a luxuriant growth of *Cladothrix* during the south-west monsoon when *Chætophora* appeared *Synedra* and *Navicula* decreased numerically during the north-east monsoon

COCANADA

Hot weather—9th June, 1931

The impounding reservoir showed luxuriant vegetation similar to Red Hills Lake—*Chara* constituting over one-half of the flora. *Nitella* and grasses were also present.

Among the lower algal forms *Phormidium*, *Lyngbya*, *Synedra*, *Anabæna* and *Nostoc* were the predominant types.

Cold weather—28th January, 1932

The water of Godavari canal was turbid, mostly due to dead fungi which had disintegrated beyond recognition.

On the bank of the canal the stones and decaying grasses were covered with 10 species of algæ, *Spirogyra* and *Mougeotia* being predominant.

The reservoir was covered with luxuriant growth of hydrophytic monocotyledons and grasses. Among the lower forms *Amphora*, *Anabæna* and *Synedra* were present which gradually decreased in number as the water reached the outlet.

On the border of the reservoir the decaying grasses harboured *Spirogyra*, *Crenothrix*, *Mougeotia*, *Phormidium*, *Oscillaria*, *Nitzschia* and *Anabæna* in order of predominance.

MASULIPATAM

Cold weather—25th January, 1932

Near Anderson Bridge the water of the Kistna canal branch was muddy, the inorganic particles settling readily. The turbidity left after settlement was due to finer inorganic particles in suspension and decaying grasses.

Diatoms were few (less than 20 per c.c. of water) for they do not normally flourish in turbid waters.

Stones on the revetments of storage tanks had growth of *Oscillaria* in which *Nitzschia* were entangled. *Chætophora* was found in tiny patches.

TABLE IX

Genera	ORGANISMS IN STORAGE TANKS PER C.C.	
	Surface water	Water at 5′ depth
A. Anabæna	62	76
A. Nostoc	172	204
C. Navicula	152	178
C. Nitzschia	176	512
C. Synedra		8

TRICHINOPOLY

Hot weather—29th May, 1931

There was a small flow in the river Cauvery—no evidence of any *Chlorophyceæ* being present A few *Diatoms* were present

The elevated reservoir on the Rock Fort—where the Infiltration Gallery Supply is stored, chlorinated and supplied to the town—has 2 chambers A sample of the sediment from the bottom of one of the chambers showed *Amphora Navicula* and *Synedra*

KODAIKANAL

Hot weather—27th May, 1931

There was no appreciable vegetation in the reservoir such as we find in the Red Hills Lake A sample of water examined after settlement showed *Amphora* and *Navicula* as the predominant types Dead *Chlorophyceæ* and the common *Diatoms* were observed in the slimy deposits in the shallow regions of the reservoir

Cold weather—21st November, 1931

A sample of water showed 3 *Tabellaria* and 1 *Fragilaria* per litre of water—a very low figure

Stones collected from the shallow portions of the reservoir showed a variety of *Diatoms*

Diatoma, Tabellaria . Abundant

Nitzschia, Phormidium, Navicula, Cymbella, Pinnularia, Amphora, Surirella, Cocconema

KING INSTITUTE WELL, GUINDY—(Closed well in daily use)

Cold weather—16th December, 1931 and 15th January, 1932

Dead tissues of angiosperms in small quantities were observed in all examinations

Algæ were entirely absent

Hot weather—18th April, 9th May and 2nd June, 1932

Navicula, Amphora and *Lyngbya* were recorded at the rates of 16, 9 and 10 respectively per c c of water A few flagellates were also observed

North-east monsoon—25th October, 1931

Phormidium was the only alga present Numerically it was very low, being 25 filaments per litre of water Flagellates and a few rotifers were also present

KRISHNAREDDY WELL, GUINDY—(Open well in daily use)

Cold weather—19th December, 1931, January 1932 and 18th February, 1932

Spirogyra was found to vegetate on the sides of the well A few filaments of *Oscillaria* (1 in 30) were interwoven with those of *Spirogyra* *Spirogyra* died early

in January when a green felt of *Oscillaria Amphora* and *Nematodes* appeared The well water contained *Chlorella* and *Amphora* at the rate of 2 and 3 per c c of water

Hot weather—18th April, 10th May and 1st June, 1932

Water in the well assumed a greenish tinge due to *Euglena* and *Dictyosphærium*, the latter showing a count of 560 per c c of water

ADAYAR RIVER AT GUINDY

Inorganic particles and decayed and decaying grasses were always present

Cold weather—17th December, 1931, 15th January, 1932 and February 1932

TABLE X

Average counts of organisms per c c of water

Genera	Raw water	Filtered water
B Euglena	2	
C Amphora	4	
C Melosira	4	
C Navicula	12	2
C Synedra	7	

Hot weather—30th April, 31st May and 4th June, 1932

TABLE XI

Average counts of organisms per c c of water

Genera	Raw water	Filtered water
A Oscillaria	30	
B Dictyosphærium	44	2
B Pediastrum	6	
C Amphora	4	
C Navicula	43	
C Nitzschia	11	2
D Crenothrix	8	

SECTION IV

LENGTH OF LIFE OF DIFFERENT SPECIES

At Lieut.-Colonel King's suggestion, experiments were undertaken to find out the longevity of life of the commoner species found in the Red Hills Lake and attempts were made to estimate the amount of debris they add to water on their death.

Pure cultures were grown in the laboratory under laboratory conditions each in 500 c c of water As a check the life of the organisms in nature was also noted

At the start (November 1931) organisms were grown roughly in the proportion they were found in the Red Hills Lake At the end of three months the debris was removed by centrifuging and the volume noted

Chara lived for over three months and left 5 c c of debris In the lake it was observed to live for five months

Scytonema grew at a rapid rate forming thick clusters in a fortnight Then the decay of outer filaments and the growth of inner filaments went on simultaneously It left 16 c c of debris The organism was found growing in the Red Hills Lake throughout the year though its life under laboratory conditions was two months

Chætophora —It lived for six weeks 1 2 c c of debris were produced In nature also it appeared to live for about the same time

Cladothrix —It lived for four months The debris produced was 6 3 c c Growth and decay go on at a rapid rate In the lake also it lived for four months

Crenothrix —It left 1 0 c c of debris It lived a little over three months In the lake it was found to flourish for the same period

Mougeotia —It lived for a month and produced 0 4 c c of debris In nature it lived for the same period

Spirogyra and *Zygnema* — These died within a week after filament formation leaving 0 5 c c of debris In nature the filaments also appeared to live for only a fortnight

Ulothrix and *Chlorella* —These lived for a couple of months leaving 0 7 c c of debris In nature they were found for two months

Anabæna, Nostoc and *Lyngbya* —These died in three months leaving 1 0 c c of debris In the lake they lived for four months

Microcystis —It lived for over three months and left 0 5 c c of debris In nature it flourished for two months

Synedra —It lived over three months and left only 0 2 c c of the debris In nature it was found for over four months

Cœlastrum —It lived for two months and left 0 3 c c of the debris In the lake it was observed for three months

Navicula, Pinnularia and *Fragilaria* —These lived much longer than three months and left 0 8 c c of debris In the lake they were found for six months

Amphora, Nitzschia and *Tabellaria* —These *Diatoms* lived *throughout the experiment* (7 months) and left 1 0 c c of debris They were always found in nature and are known to retain their vitality for years

The importance of *Scytonema, Cladothrix,* and *Chara* in the production of organic debris in the lake is manifest.

SECTION V

Copper sulphate treatment

(1) *Method* —To estimate the quantity of copper sulphate required to kill the various organisms 250 c c of a sample was poured into each of 20 conical flasks and treated with a freshly prepared 0 01 per cent solution of copper sulphate The first 16 flasks received amounts increasing by 0 25 c c for each flask—thus in these the copper sulphate strength increased from 0 1 part per million to 1 6 parts by successive steps of 0 1 part The next three flasks received amounts to make the strengths 1 8, 2 0 and 2 5 parts per million The last or 20th flask was kept free from copper sulphate as a control

After 24 hours each jar was examined and the condition of the organisms recorded

(2) A *Red Hills Lake water in the south-west monsoon* —19th July, 1931

No 1 *Navicula* was dead All other organisms were normal

No 2 *Navicula* and *Asterionella* dead Other organisms normal

No 3 *Navicula, Asterionella* and *Ulothrix* dead Other organisms normal

No 4 *Navicula, Asterionella, Ulothrix, Crenothrix* and *Cladothrix* dead

Nos 5 to 14 Same as No 4

No 15 *Nitzschia* dead, in addition to those in No 4

No 16 *Synedra* dead, in addition to those in No 15

Nos 17 and 18 Same as No 16

No 20 *Navicula, Asterionella, Ulothrix, Crenothrix, Cladothrix, Nitzschia* and *Synedra* all living

These flasks were left over for another 48 hours and examined again There was no further change

Therefore, in parts per million, the doses required to kill the organisms were *Navicula* 0 1, *Ulothrix* 0 3, *Asterionella* 0 2, *Crenothrix* 0 4, *Cladothrix* 0 4, *Nitzschia* 1 5, *Synedra* 1 6

Beggiatoa did not die even when the copper sulphate was added at the rate of 2 5 parts per million

Higher doses at the following rates were applied —

1	7 5 c c	of 0 1 per cent	solution	making	3	parts per million
2	10 0	,,	,,		4	,, ,,
3	12 5	,,	,,		5	,, ,,
4	15 0	,,	,,		6	,, ,,
5	17 5	,,	,,		7	,, ,,
6	20 0	,,	,,		8	,, ,,

The organism was killed in flask Nos 5 and 6 By treating it with doses 6 1, 6 2, 6 3 6 9, it was found that it was killed by 6 2 parts per million of copper sulphate

B *Red Hills Lake*

Cold weather —6th January, 1932

In parts per million, the doses required to kill the organisms were *Nitzschia* 1 3, *Synedra* 1 5, *Melosira* 0 7, *Fragilaria* 0 1, *Nostoc* 0 2, *Ulothrix* 0 3, *Chlorella* 0 3, *Crenothrix* 0 1, *Cladothrix* 0 1

South-west monsoon —19th July 1931

Chlorella 0 3, *Ulothrix* 0 3, *Asterionella* 0 2, *Navicula* 0 1, *Nitzschia* 1 5, *Synedra* 1 5

North-east monsoon —16th October, 1931

Chlorella 0 3, *Ulothrix* 0 3, *Amphora* 1 0, *Asterionella* 0 2, *Navicula* 0 1, *Nitzschia* 1 5, *Synedra* 1 5, *Tabellaria* 0 8, *Cladothrix* 0 4, *Crenothrix* 0 4

KILPAUK

Cold weather —19th January, 1932

Clathrocystis 0 3, *Fragilaria* 0 1, *Navicula* 0 1, *Nitzschia* 1 3, *Synedra* 1 5

Hot weather — 27th May, 1932

Anabæna 2 2, *Oscillaria* 0 3, *Microcystis* 0 4, *Mougeotia* 0 2, *Navicula* 0 1, *Nitzschia* 1 5, *Cladothrix* 0 4, *Crenothrix* 0 1

South-west monsoon —15th August, 1931

Clathrocystis 0 3, *Nostoc* 0 2, *Phormidium* 0 3

North-east monsoon —20th October, 1931

Phormidium 0 3, *Melosira* 0 7, *Navicula* 0 1, *Nitzschia* 1 5, *Synedra* 1 5

APPENDIX I

NOTES ON MEDIA

Most organisms grow well in ordinary water and nutrient solutions need be used only to hasten their growth Unless otherwise mentioned distilled water should be used in making up the following solutions and since algæ are very sensitive to copper, only glass distilled water should be used —

1 STOCK SOLUTION

Calcium nitrate	50 g
Potassium nitrate	12 ,,
Sodium chloride	7 ,,
Pot hydrogen phosphate	13 ,,
Magnesium sulphate	25 ,,
Distilled water	2 litres

A dilution (1 in 50) is used for the growth of most of the algæ and particularly for *Rivularia* and *Cladothrix*

2 POTASSIUM HYDROGEN PHOSPHATE

0 2 per cent solution in tap-water

This can be used for growing *Cyanophycea*, e g , *Anabæna, Cylindrospermum, Nostoc* \ 50 per cent dilution is used for *Diatoms* like *Tabellaria, Synedra* and *Fragilaria*

3 MOORESCHE SOLUTION

Ammonium nitrate	0 05	per cent
Magnesium sulphate	0 02	,,
Pot hydrogen phosphate	0 02	,,
Calcium chloride	0 01	,,
Ferrous sulphate	traces	

The solution promotes the growth of filamentous forms, e g , *Phormidium, Lyngbya* and *Scytonema*

4 CHODAT'S MEDIUM

Calcium nitrate	0 1	per cent
Pot hydrogen phosphate	0 03	,,
Magnesium sulphate	0 03	,,
Potassium chloride	0 01	,,
Ferrous sulphate	traces	

pH to be adjusted to 5 3

Useful for the growth of unicellular forms like *Chlorococcum*

5 BENECKE'S MEDIUM

Calcium nitrate	0 05	per cent
Magnesium sulphate	0 01	,,
Pot hydrogen phosphate	0 02	,,
Ferric chloride	traces	

For unicellular forms like *Chlorococcum* and colonial forms like *Cœlosphærium*

6 BRISTOL'S SOLUTION

Calcium nitrate	0 15	per cent
Magnesium sulphate	0 05	,,
Pot hydrogen phosphate	0 02	,,
Calcium chloride	0 01	,,
Ferrous sulphate	traces	

For *Ulothrix*

7 KNOP'S SOLUTION

Potassium nitrate	1 g
Potassium phosphate	1 ,,
Magnesium sulphate	1 ,,
Calcium nitrate	4 ,,
Ferric chloride	traces

A dilute solution (1 in 20) is used for the growth of *Hormidium*, *Debarya*, *Nodularia*, *Glœotrichia* and *Pandorina*

APPENDIX II

SYSTEMATIC POSITION OF THE GENERA STUDIED

ORDER *Isokontæ*

Volvocales Sub-order

Genera *Chlamydomonas*, *Eudorina*, *Volvox*, *Tetraspora*

Chlorococcales Sub-order

Chlorococcum, *Chlorella*, *Pediastrum*, *Ankistrodesmus*, *Dictyosphærium*, *Scenedesmus*, *Cœlastrum*

Ulotrichales Sub-order

Ulothrix, *Hormidium*

Chætophorales Sub-order

Chætophora, *Pleurococcus*

Conjugatæ Sub-order

Debarya, *Zygnema*, *Spirogyra*, *Mougeotia*, *Desmidium*, *Staurastrum*

ORDER *Heterokontæ*

Heterotrichales Sub-order

Tribonema

ORDER *Bacillariales*

Centricæ Sub-order

Melosira

Pennatæ Sub-order

Tabellaria, *Diatoma*, *Fragilaria*, *Synedra*, *Asterionella*, *Navicula*, *Plemosigma*, *Cymbella*, *Amphora*, *Nitzschia*, *Surirella*

ORDER *Euglenineæ*

Euglena

ORDER *Myxophyceæ*

Cœlosphærium, *Microcystis* (*Clathrocystis*) *Glœocapsa*, *Chroococcus*, *Oscillatoria*, *Phormidium*, *Lyngbya*, *Nostoc*, *Anabæna*, *Cylindrospermum*, *Scytonema*, *Calothrix*, *Rivularia*, *Glœotrichia*

ORDER *Charæ*.

Chara, *Nais*

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STUDIES ON CHOLERA BACTERIOPHAGE

Part I

GENERAL TECHNIQUE

BY

IGOR N ASHESHOV, INNA ASHESHOV, SARANJAM KHAN AND M N LAHIRI *

(From the Bacteriophage Inquiry, Indian Research Fund Association, Patna)

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- Introduction
- Media
- Some of the special appliances required
- Equipment for filtration
- Collection of material
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  - Short cut method of ascertaining the presence of bacteriophage
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INTRODUCTION

THE novelty of the question, the unexpected phenomena with which *Protobios bacteriophagus* manifests itself have made many an investigator doubt its living nature Even the unconquerable logic of d'Herelle's argumentation does not dispel all doubt During ten years of constant work on bacteriophage I have myself

* These papers are written by the senior author (I N A) The plural 'we' refers to the studies done by some members of the staff together The singular 'I' is used where the senior author is personally responsible for an opinion expressed, observation made, or experiment performed

only too often doubted its living nature, but each experiment invariably led to the one conclusion—bacteriophage cannot be anything but a living being

In this paper we do not intend to bring forward any special experiments to prove the above statement Its object is essentially practical, to introduce more definite or new methods in our study and in application of bacteriophage

The definite recognition of the living nature of bacteriophage made it clear that our previous technique of work with it was not satisfactory It became evident that on the one hand we ought to proceed with the study of bacteriophage in exactly the same manner as with the study of bacteria, and, on the other hand, we must take into consideration its ultra-microscopical nature, its filtrability, and accordingly introduce adequate modifications in the usual bacteriological technique

Up to now, however, these elementary principles have not generally been recognized and applied in laboratories working on bacteriophage Some instances may be cited where the fallacies of the common technique are evident

The lysis of bacteria produced by a filtrate of, say, a stool emulsion merely indicates that this filtrate contains bacteriophage, or, what is more probable, a mixture of many bacteriophages The lysis of bacteria has with bacteriophage the same meaning as turbidity in broth with bacteria—it indicates its presence But we know as yet nothing about the bacteriophage present and, in my point of view, it would be as great a mistake to study such a filtrate as 'a bacteriophage' as to study a broth culture of a sputum, for example, as a biological entity By making the subsequent passages of the filtrate on one kind of bacteria we may eliminate the majority of bacteriophages present in the original filtrate It is like making a passage of sputum through mice to obtain a culture of pneumococci We have no right to treat the blood of the mouse as a pure culture of pneumococci—it may contain different types of them or be contaminated with other micro-organisms, such as streptococci In that way, if we recognize the living nature of bacteriophage, we have no right to treat our filtrate as a pure culture of bacteriophage and to study it as an entity There can be present different races of bacteriophage growing on the same bacteria, as I have already shown (Asheshov, 1924) In the study of bacteriophage we must use the pure-line cultures as we do in the study of bacteria Also (Asheshov, 1930) there exist different types of bacteriophages active against the same micro-organism, which, being antigenically different, possesses also other different properties, often of opposite character It is evident that we cannot study the character of bacteriophage in such a mixture of types, but only the properties of the mixture, otherwise our results will be inaccurate and our conclusions misleading For instance, the Type A of choleraphage in the presence of other types of choleraphage multiplies during the first 18 hours, after which its number tends to diminish On the contrary, Type B grows more slowly, but continues to do so longer for, say, 36 hours, while Type C grows slower and longer still

Therefore, if we disregard the presence of different types and study our mixture as 'a bacteriophage', we will conclude that this 'bacteriophage', if filtered early, lyses bacteria quickly, but allows the development of secondary growth, while if filtered later it lyses bacteria more slowly, but the lysis is more permanent In this way we ascribe to bacteriophage a definite property which actually it does not possess What actually happens is that only the relative number of different types of bacteriophage changes, changing the character of the mixture but not of bacteriophages themselves Dozens of such examples could be cited

The clearings produced by bacteriophage in the bacterial layer are its colonies and, as in case of bacterial colonies, different bacteriophages show different morphology of these clearings* This morphology must be studied in the same way as we study the morphology of bacterial colonies Applying the usual method of isolation we can obtain different races of bacteriophage in pure culture These cultures for proper biological study must be maintained in a pure state and protected from contamination by the other races of bacteriophage This has seldom been recognized We must make an allowance for the filterable† nature of bacteriophage During certain moments of the work, bacteriophage cultures are more easily contaminated than bacterial cultures The majority of investigators paid attention only to the 'sterility' of the bacteriophage cultures They did not care what happened to the culture before filtration the filter will eliminate the possible bacterial contamination and attention was given only to the liquid on the other side of the filter But if we contaminate a culture of bacteriophage with another one, both will pass through the candle and our filtrate, from the point of view of ultra-purity, will still be a contaminated one, unsuitable for proper study For instance, the usual technique was to prepare a Kiselguhr-covered filter in a non-sterilized funnel, the suspension of Kiselguhr being made with tap-water Bacteriophage cultures, being 'sterile', are usually handled in such a manner that the laboratory itself is heavily contaminated with different bacteriophages To use such a method in working with bacteria would be considered the greatest offence against elementary rules, yet it is freely applied in work with bacteriophage Lack of exercising the necessary care during every stage of work with bacteriophage cultures has resulted in curious statements, such

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* Since my first publication in English mentioned, I have insisted on using the term 'clearings' in reference to the areas of bacteriophage action produced in bacterial layer It seems to me that the term 'plaques', adopted by d'Herelle and Smith and accepted by the majority of authors, is not descriptive and even confers a wrong idea as to the structure of a clearing 'plaque' means something elevated above the surface, rather than an 'eaten out' area Therefore, until a still better expression can be found, I will continue to use the word 'clearings'

† Here I use this term in its ordinary meaning = capability to pass through the usual porcelain filters The use of graduated collodion membranes necessitates revision of this term

as obtaining bacteriophage from doubly distilled and autoclaved water In our work we have tried to introduce strict rules which helped us to maintain our cultures in an ultra-pure state

It has very seldom been taken into consideration, although well known, that bacteriophage cultures can contain quite different number of bacteriophage corpuscles But very seldom in experiments where the number of bacteriophages was of importance, has any attempt been made to use a definite number of corpuscles as opposed to a definite quantity of filtrate, which indicates nothing So, for instance, the estimation of comparative virulence of two bacteriophages would be made with one drop of each filtrate in 10 c c of bacterial emulsion and a conclusion would be drawn that one bacteriophage was of poorer virulence than the other, not considering the possibility that the reverse might be true and that one drop of the first filtrate contained perhaps one million times less corpuscles than one drop of the second Whenever necessary we tried to use a definite number of bacteriophage corpuscles and not a definite quantity of the liquid

The influence of the reaction of the fluid medium on the optimal development of bacteriophage has long been recognized Media with carefully adjusted reaction were used, but attention was seldom paid to the changes which occur in the course of cultivation With the majority of bacteria these changes are already considerable at the early stages of cultivation and only too often reach the point where the development of bacteriophage or its consequent conservation suffer To all liquid media in our laboratory which are liable to change their reaction during cultivation we add one of the sensitive indicators, such as phenol red, for direct reading and correction of the reaction during cultivation

In this paper I shall go into details of the technique which was developed during my previous studies on bacteriophage and suitably adapted to the investigation of this Inquiry into choleraphage It aims at obviating the fallacies of which some have been mentioned above, but it is still far from ideal and efforts to improve it have our daily attention

The principle of handling the bacteriophage as a culture of a living being applied successfully in practice showed once more that the living nature of bacteriophage is not a hypothesis, not a theory, but a fact If bacteriophage is devoid of life, our technique would have served as a *reductio ad absurdum*—it would fail But practice shows that bacteriophage handled as a living being behaves as such and confirms our expectations

The methods developed during the work of this Inquiry were made to suit the work on choleraphage, but their principles, in my view, are applicable when working with other bacteriophages as well

Media

Papain digested meat media Concentrated Broth

This was found to be one of the best and cheapest media for general use The following method was worked out in our laboratory and has been used successfully during the last three years —

One thousand grammes of well-chopped meat is thoroughly mixed with 4 litres of distilled water Fifteen grammes of powdered papain* is well emulsified in a little water and added to the meat The reaction is adjusted and maintained throughout the digestion as far as possible near pH 7 0 To obviate the complicated use of a colorimeter to control the reaction we use the simple ' drop ' method on a white porcelain plate or on a glass plate placed on white paper, one drop of medium is mixed with one drop of 0 02 per cent phenol red One soon learns to recognize the delicate salmon colour of pH 7 0 All is put in a large saucepan (*degchi*), placed within another with water, making a water-bath The temperature of the mixture is brought to 70°C and so maintained for three hours By that time the digestion for our purpose is complete The digest is filtered through ' agar paper ' (so-called type Chardin or 1117½ S and S), the reaction adjusted to pH 8 4, and sterilized at 115°C for 20 minutes During the sterilization a coagulum forms which clears the liquid considerably and helps the subsequent filtration This constitutes the Concentrated Papain Broth

Concentrated Peptamin Broth

I have used this medium with good results since 1921 (Asheshov, 1924) Though more complicated and expensive than papain media it has the valuable advantage of bringing out particularly well the morphology of bacteriophage clearings and is therefore used for agar in Petri dishes

One thousand grammes of well-chopped meat are thoroughly mixed with 2 litres of 1 per cent HCl in ' distilled ' water and 0 5 to 1 g of pepsin Ph Br IV (1 2,500) (Merck) is added It is left at 37°C for 24 hours No preservative is usually required but in hot weather in India, when meat is thoroughly contaminated with micro-organisms that would grow even in such an acid medium, addition of chloroform (about 15 c c for 4 litres) is advisable

Next day, to the jelly-like mass is added ammonia up to slightly alkaline reaction to the litmus The mass becomes fluid It is then brought to boiling point It must coagulate well, leaving the liquid quite transparent If it does not coagulate properly, more ammonia is cautiously added (It is curious that the

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* Unpurified dried juice of *Carica papaya* Its use for bacteriological purposes was first suggested by Martin (1927) It can be obtained from F J Holloway, Esq , Trafford Hill, Golagedere, Ceylon

coagulation is more complete in an alkaline medium) The liquid is now cooled quickly and strained through muslin The liquid part is sterilized and put aside as the 'Peptone Part'

To the coagulated meat collected on the muslin about 200 g of chopped liver, 2 litres of distilled water and 0 5 g of *Pancreatinum absolutum* (Merck) are added The reaction is made distinctly alkaline by the addition of ammonia (litmus paper clearly blue) Some 15 c c of chloroform is added as a preservative The whole mixture is left for about one week or longer, when the digestion is complete and the liquid is rich in tryptophane This constitutes the 'Amino Part'

Both peptone and amino parts are mixed without preliminary filtration, the reaction is adjusted to about pH 7 2 by addition of ammonia, the whole is sterilized and then cooled quickly to promote sedimentation It cannot be filtered as the undigested particles are very fine and clog the filter immediately The next day it is already possible to decant the larger part of the liquid The rest is mixed again, sterilized and left to settle until required The decanted liquid constitutes the 'Concentrated Peptamin Broth'

Preparation of routine media

Dilution of concentrated broth —It is the routine practice of the majority of laboratories to estimate the dilution of broth by the quantity of meat used—every kilogram of meat giving so many litres of broth It is evident that in this way every batch of the broth is of different nutrient properties, the difference being sometimes considerable Some laboratories have recognized this fallacy and tried to introduce some more definite standard of dilution, aiming at obtaining a more constant composition of their media Considering the amino part of the broth as the most important, the methods of estimation of amino acids were introduced At the beginning of our work we also diluted our concentrated broths down to a certain concentration of amino acids However, it was soon recognized that it is not only the amino acids but other organic matter as well that play an important part in determination of the nutrient property of our media Therefore it was considered more logical to have recourse to an 'estimation of all the organic matter' contained in the concentrated broth and then to dilute accordingly The *Kjeldahl's* method of nitrogen determination is too complicated and requires too much time and attention for everyday use On the contrary, the method of determination of oxidizable matter, based on the same principles as that used in water examination, was found to give a very fair estimation of the nutrient properties of the medium It was worked out in the following manner —

Determination of oxidizable matter —Reagents necessary —

1 Standard oxalic acid solution containing 7 88 g per litre
2 Standard potassium permanganate solution—4 g per litre
3 Sulphuric acid solution—1 in 4

On a chemical balance weigh accurately 7 88 g of chemically pure oxalic acid and dissolve it in exactly one litre of distilled water

In another flask dissolve approximately 4 g of potassium permanganate in 900 c c of distilled water

Into a 500 c c flask put about 200 c c of distilled water From a burette measure into this flask 10 c c of permanganate solution and add about 10 c c of sulphuric acid solution Bring to boiling point and maintain it boiling for exactly (by stop watch) 5 minutes From another burette add 10 c c of standard oxalic acid solution and continue to add it cautiously drop by drop until the pink colour in the flask disappears Note the amount of oxalic acid solution used This will indicate how much the prepared potassium permanganate solution must be diluted to be equivalent to standard oxalic acid solution If, for instance, 11 2 c c of oxalic acid solution were used, then every 10 c c of permanganate solution must be brought up to 11 2 c c This will constitute the standard potassium permanganate solution

It is recommended that this preliminary titration be repeated before each examination of the broth, if done on different days, beginning with titration of the solution containing 4 g of potassium permanganate in 900 c c and diluting it each time afresh as indicated

Determination of the oxidizable matter in the broth —

Prepare accurately a 1 10 dilution of the broth in distilled water Add 1 c c of it to about 200 c c of distilled water in a 500 c c flask Add 10 c c of sulphuric acid solution and from a burette run in exactly 10 c c of standard potassium permanganate solution Boil for exactly 5 minutes While still hot, add accurately 10 c c of oxalic acid solution, which will completely decolorize the mixture in the flask Add cautiously to this decolorized liquid the standard potassium permanganate solution until first traces of pink are noticed The amount of potassium permanganate solution required indicates the amount of it spent on oxidization of the organic matter in 0 1 c c of concentrated broth taken for examination As 1 c c of standard potassium permanganate solution contains 1 milligram of oxygen, it follows that each c c of this solution spent in titration of our sample indicates that every 100 c c of our concentrated broth contain such an amount of organic matter as to require 1 gramme of oxygen for oxidization Colloquially we say that each 1 c c of standard potassium permanganate solution indicates '1 per cent of oxidizable matter' in our concentrated broth

This titration indicates the amount of the total organic matter, including amino-acids, peptones, proteoses, etc , present in our medium As not only amino-acids serve as the food for bacteria, it is evident that in this way we get a much better idea of the true nutrient value of our medium than in the case of determination of the amount of amino-acids alone Experience proves this For instance we have on our log batches of broth with '1 8 per cent oxidizable matter', but of

which the content in amino-acids varies from 0 21 per cent to 0 37 per cent Diluted to the standard 'oxidizable matter' content both batches gave broth of practically the same nutrient property, though the content in amino-acids was 0 058 per cent and 0 103 per cent respectively Diluted to the standard amino-acids content, the first batch proved to be unnecessarily concentrated

However, as for some investigations it is of importance to follow the amino-acids content, I am giving here the method of their determinations, as used in our laboratory —

Quantitative determination of amino-acids —This method is based on that of Sorensen as developed by Brown (1923) and slightly modified to suit our requirements

1 Take 500 c c of commercial formalin Add 5 c c of 1 per cent phenol red Add strong (15 per cent) NaOH solution until the liquid does not become more red on further addition of alkali Leave it until next day If during this time the formalin becomes again acid, add more alkali Filter

2 Take 5 c c of standard normal solution of NaOH, add 1 c c of 1 per cent phenol red and dilute with distilled water up to 100 c c, thus obtaining N-20 solution

3 Take three small beakers, not necessarily of the same diameter

Beaker I Put some 50 c c of prepared formalin Adjust its reaction to approximately pH 7 8 In my opinion this is the most sensitive point of phenol red

Beaker II Put 4 c c of distilled water Add exactly 1 c c of concentrated broth, and 0 5 c c of 0 1 per cent solution of phenol red

Beaker III From beaker I pour formalin to the *same level* as the level of the liquid in beaker II

4 Into beaker II add alkali or acid until its colour is exactly the same as that of the formalin in beaker III From beaker I add 8 c c of formalin to beaker II Also from beaker I add a sufficient amount of formalin to beaker III to bring it to the same level as in beaker II

5 After addition of formalin into beaker II the liquid in it becomes acid due to the combination of formalin with amino-acids By means of a 1 c c pipette add sufficient amount of standard N-20 solution of NaOH to bring its colour to that of beaker III

6 Multiply the amount of N-20 NaOH used for the last titration by 70 It will give the amount of amino-acids in milligrams in 100 c c of concentrated broth

After numerous trials we found that the best media are obtained if we dilute our concentrated broth ('Papain' or 'Peptamin') to '0 5 per cent of oxidizable matter' content for broth and to '0 75 per cent of oxidizable matter' content for agar Lower content in oxidizable matter results in poorer growth, while further increase of it does not improve the quality of the medium noticeably

Broth—The necessary amount of concentrated broth is first filtered through paper and diluted to the '0 5 per cent oxidizable matter' content with distilled water 0 5 per cent sodium chloride is added Reaction is adjusted to pH 8 0 0 1 c c of 1 per cent phenol red solution is added for every litre and the whole is sterilized at 115°C for 20 minutes It is then filtered, if necessary, through Kiselguhr covered filter-paper, until crystal clear, and then distributed into test-tubes or flasks and sterilized again

Agar for plates—The required amount of peptamin concentrated broth is diluted to contain '0 75 per cent oxidizable matter', the reaction is adjusted to pH 7 4, the broth is sterilized and filtered repeatedly until perfectly clear This often saves the necessity of subsequent clearing the agar with egg and always gives a more transparent medium, so essential for the study of bacteriophage clearings No salt is added 1 1 per cent of China grass (agar-agar) *and no more* is added I have often heard the objection that such an agar cannot be used in a hot climate Such an objection does not hold true The agar sets at about 40°C—41°C It starts to melt in the neighbourhood of 65°C Even in the hottest time of the year in India the temperature during the night only exceptionally rises higher than 37°C (98°F) It is true that the agar under those conditions takes many hours to set Our routine practice is to pour the plates in the afternoon and to leave them to set overnight Next day they are of normal consistency The main precaution to obtain a good, elastic and not brittle agar is not to heat it too many times Three sterilizations (to dissolve the agar-agar, to coagulate the egg for clearing, and to sterilize after filtration) are almost the limit After a fourth sterilization there is already a risk that it will be too brittle When I am preparing media myself I use 1 per cent agar The additional 0 1 per cent for the agar prepared by media-makers is added for safety's sake only a 0 9 per cent agar is already too soft for plating

Such a light concentration of agar must be insisted on because it is absolutely essential for the study of the morphology of bacteriophage clearings 1 25 per cent agar is less satisfactory Over 1 5 per cent agar is almost useless for the purpose Besides, 1 1 per cent agar is very fluid while hot and is easily filtered through Chardin paper, giving a clear medium, essential for morphological studies

The agar is dissolved by steaming for 20 minutes, cooled to 60°C and the whites of one or two eggs added In some laboratories I have seen as a routine that the white of egg is added along with China grass before the latter is dissolved In that way it is impossible to obtain a clear agar as the egg coagulates before the grass is dissolved and fails to catch all the impurities in the coagulum To ensure a good coagulation the dissolved agar with the egg added must be heated at least half an hour While hot it is filtered through 'agar paper' (Chardin or S and S No 1117½) and not through gauze and cotton-wool This last method gives a large amount of waste but fails to give as clear a solution as

filter-paper The filtered agar is distributed into 200 c c flasks and sterilized Before pouring the plates it must not be sterilized but only heated sufficiently to be dissolved

Agar for slopes —Papain concentrated broth is used It is filtered through paper, if necessary even through Kieselguhr-covered paper, until perfectly clear It is diluted down to '0 75 per cent oxidizable matter' content, reaction is adjusted to not more than pH 7 0, and 2 c c of 1 per cent cresol red is added to every litre No salt is used 1 5 per cent of China grass is added and the whole is sterilized at 120°C for 20 minutes, then filtered through 'agar paper' without clearing with egg If the broth was clear, the agar will be also sufficiently clear for slopes

Agar for Legroux tubes —This is prepared from peptamin broth in the same way as for plates except that 1 5 per cent China grass is used With due precaution in drying, a 1 1 per cent agar also can be used

Bile agar —This is used for isolation of the cholera vibrio It is prepared from papain broth, diluted down to '0 75 per cent oxidizable matter' content, 0 5 per cent salt and 0 5 per cent sodium choleate P G I (wrongly called 'taurocholeate') (Merck), are dissolved in it, reaction adjusted to pH 8 2 and the whole filtered through paper 2 5 per cent of China grass is added, as sodium choleate makes the agar brittle It is sterilized to dissolve China grass, cleared with egg, filtered through agar paper and distributed into flasks for plates or directly into Legroux tubes and sterilized This simple agar gives an excellent differentiation of cholera and allied vibrios

Tap-water —This is used for dilutions of bacteriophage when no subculture is necessary Tap-water is first boiled, cooled, filtered, 0 2 c c of 1 per cent solution of brom cresol purple is added to distinguish it from normal saline, and it is distributed by exactly 10 c c quantities into 8″×1″ tubes and sterilized

Peptone-free medium —For some investigations it is necessary to use 'peptone-free' media but containing sterilizable non-digested protein For this purpose we use the 'Lymphe' medium of Tricone (1930)

Sodium phosphate	0 5 g
Calcium biphosphate	0 5 „
Sodium chloride	5 0 „
Egg yolks	2 to 3
Distilled water	1,000 c c

Adjust the reaction to pH 7 0 Sterilize at 120°C for 20 minutes Cool quickly Filter first through 'agar paper', then through ordinary filter-paper Distribute into test-tubes and sterilize at 105°C for 15 minutes The medium must be slightly opalescent

Peptone water —For peptone water we use only Difco peptone as giving best and constant results One per cent peptone and 0 5 per cent NaCl are dissolved in distilled water and the reaction adjusted to pH 8 0 with NaOH solution It is filtered through ordinary paper, distributed into tubes and sterilized at 115°C for 15 minutes It is important to avoid a second sterilization as it gives an inferior medium for work with bacteriophage

For preparation of all media I strongly recommend the use of distilled water throughout Only in this way it is easy to obtain crystal clear media so essential in work with bacteriophage Tap-water can be used only if it is particularly soft Only for preparation of large quantities of bacteriophage do we use Ganges water, which before being used is first boiled, cooled, left to sediment overnight and then filtered until absolutely clear

For adjusting the reaction of all media, and particularly for peptamin agar, I recommend the use of ammonia in preference to NaOH, as ammonia salts are advantageous in bringing out the morphological peculiarities of bacteriophage clearings The exception is made only in the case of peptone water where NaOH solution is to be preferred as ammonia gives less constant results

Some of the special appliances required

Test-tubes —I strongly recommend for use in bacteriophage work test-tubes of large size We use 8″×1″ with 10 c c and 20 c c of liquid media d'Herelle pointed out that dysentery and staphylococcus bacteriophages develop better, produce quicker lysis, and gain sooner in virulence if they are cultivated in hyperaerobic conditions We found that choleraphages also multiply quicker and give larger resulting number of corpuscles in hyperaerobic conditions Therefore we applied hyperaerobiosis not only when producing large amounts of bacteriophage but in everyday practice It was for that purpose that the large tubes were introduced They are more convenient in work with loops and spreaders (*see* under 'Platings' and 'Type test') These tubes are held in especially designed stands (Plate XLVI, fig 1) which permit keeping the tubes in a slanting position (as well as in the upright one) throughout the cultivation This gives a very large surface and a thin layer of the liquid, promoting considerably the action of bacteriophage

For agar slants and for peptone water we use 7″×0 7″ tubes We found them better than the usual 6″ tubes in preventing contamination

Larger Petri dishes than the usual are recommended We use 120 mm ×20 mm this size being more convenient for plating and spreading For the study of morphology of bacteriophage clearings 50 c c of agar is poured into the dish, giving a rather thick layer essential for that kind of work For routine purposes 40 c c is sufficient

Legroux tubes *—These were found exceptionally useful for work in India for many purposes Two varieties are used the large, measuring 17 cm ×4 5 cm (L N 1791) (Plate XLVI, fig 2), and the smaller, 17 cm ×2 7 cm (L N 1560) They are used with both agar and liquid media These are filled with 20 c c and 15 c c of liquid media or 28 c c and 19 c c of agar respectively With liquid media they give a very thin layer and very large surface With solid media they give a very convenient surface for isolation of bacteria or for obtaining them in large quantity These tubes are particularly useful in field work, where they advantageously replace Petri dishes, being protected from contamination Tubes with positive results can be kept for a very long time, sealed if necessary, to obtain cultures of first transfer For that reason we prefer them to Petri dishes even for routine isolation of vibrios not only in the field but in the laboratory as well

When plugging them, it is advisable to insert along with the cotton-wool plug a fourfold slip of agar paper to collect the water of condensation accumulated on the walls of the tube Before use they are put for a couple of days into an incubator, neck down, to dry If 1 1 per cent agar is used in Legroux tubes, they must be first dried for two days in the horizontal position, agar layer up, then in the upright position, neck down

In case of emergency, Legroux tubes can be replaced by flat rectangular medicine bottles of 8 oz capacity

When plugging flasks, tubes, etc , I strongly recommend the use of non-absorbent cotton-wool It is more economical, is a better protective against contamination and does not get wet in sterilization, unless the latter is done carelessly

Equipment for filtration

Aluminium funnels and covers —For the preliminary filtration through Kiselguhr-covered filter-paper the glass funnels were replaced by aluminium ones We use funnels of 2 6″ (6 5 cm) top diameter, 2 2″ (5 5 cm) height of the body, 60° angle and 1 5″ (4 cm) long stem† They are used with a pleated filter-paper disk of 12 5 cm diameter (S & S No 588) and can conveniently hold 20 c c of liquid The funnels with folded filter in them are mounted on an ordinary test-tube, covered with paper and sterilized (Plate XLVI, fig 3) I do not advise the use of glass funnels As they have to be sterilized before and after use they crack and break too easily

The funnels are provided with special covers to protect the contents during filtration from flies and possible splashing from nearby funnels

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* Obtainable from Etablissements Leune, 28-bis rue Cardinal Lemoine, Paris, France

† Funnels and covers for them were made for us by Aluminium Manufacturing Co , Ltd , 14, Clive Street, Calcutta

Kiselguhr suspension—The trials showed that ½ per cent suspension of Kiselguhr* gives the best film on the filter paper bacterial emulsion filters through it quite quickly, giving generally a crystal clear filtrate, though a heavy stool emulsion probably will require double filtration Such a suspension is prepared in advance in 500 c c flasks and sterilized

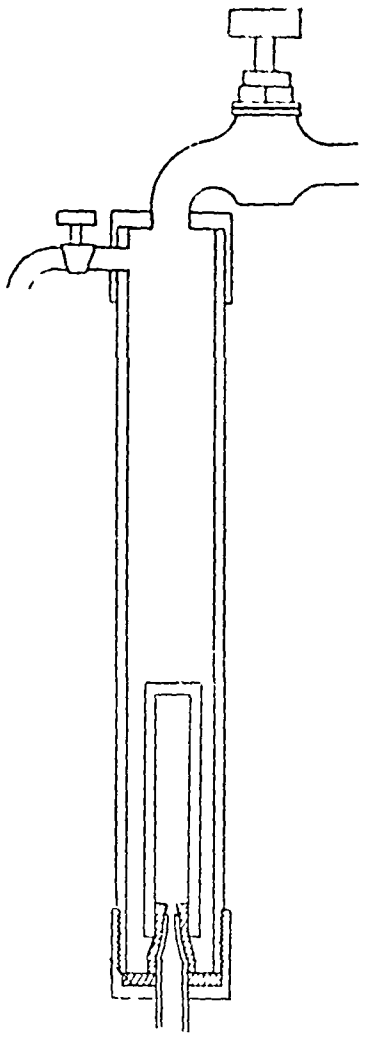
Cylindrical funnels—The bulbous funnels usually supplied with Martin filtering flasks were found unsatisfactory They crack easily during sterilization and are difficult to clean Their stem is too thin and the small india-rubber cork gets loose after a short time The funnel made for me in India† proved to be more convenient in use This funnel consists of a glass cylinder 1 2″ (3 cm) diameter, 3 5″ (9 cm) high As we use 1 25″ high india-rubber stoppers, the stem is made 2 5″ (6 5 cm) long It is ⅜″ (9 mm) thick, with tapering end, the point being about $\frac{3}{16}$″ (4 mm) in diameter Instead of the cork at the end to fit the candles, we use india-rubber vacuum tubing 10 mm thick with 1 mm bore When put on the stem of the funnel it makes a long, slightly conical stopper Being a little longer than the stem, it gives a soft end, easily collapsible to fit even the smallest bore of filter candle (Plate XLVI, fig 4)

Candles—Throughout routine work L3 Chamberland candles were used, mainly of 75 mm ×15 mm size, though for filtering larger quantities of liquid the F or B (*embasse a teton*) were used (Plate XLVII, fig 5) Each new candle is washed thoroughly with a brush and dried Each candle is given a current number, which is written on the rough surface of the candle with wax pencil and then lightly burned to destroy the wax and to leave a brick-coloured figure The used candles are boiled immediately after filtration The following detail prolongs the life of a candle very considerably Inside the saucepan in which the candles are boiled, a line is drawn 2 5″ (6 5 cm) from the bottom and water is always poured up to this level When the candle, with the non-absorbent cotton-wool round its neck, is put into the water, it will float in upright position, without being filled with water Water will gradually percolate into the candle through the walls washing away the organic matter, which no boiling could remove effectively After boiling the candles are thoroughly dried in an incubator and then burned to dull red-heat in a muffle oven for 45 minutes The burning is indispensable Beard (1931) showed that boiling of the candles even for 40 minutes does not always destroy bacteriophage inside the candle I have found 'Muffle Furnace, Multiple Unit, Electric' with enclosed rheostat ('Cenco' Catalogue No 6372) very convenient in use It takes 45 minutes to bring the candles to dull red-heat, and during the total time of 1½ hours this furnace

* I prefer to use the 'Kiselguhr, white, for filtration' (Baird and Tatlock), though the dark variety used in France is also very good

† By the Scientific Instrument Co , Calcutta

consumes less than two units of current After the candles are cooled, the small ones (L3) are ready to be mounted The big ones (F and B) are better washed through to remove the inorganic ash from inside the pores and then tested for soundness A very convenient way to wash these candles is to filter some water through them, using Chamberland filter No 28 (Plate XLVII, fig 5) A similar apparatus can be made out of 1" water-pipe (*see* Text-figure)



TEXT-FIGURE

Testing candles —A testing apparatus can be easily arranged in the following way —

Parts required a pressure tank with pressure gauge used for large petrol or kerosene incandescent lamps, a motor car foot-pump, a large pressure gauge fixed on a T-tube with a T-stop cock at the joint All are connected with heavy pressure tubing A well-moistened candle is connected to the T-tube and put under water.

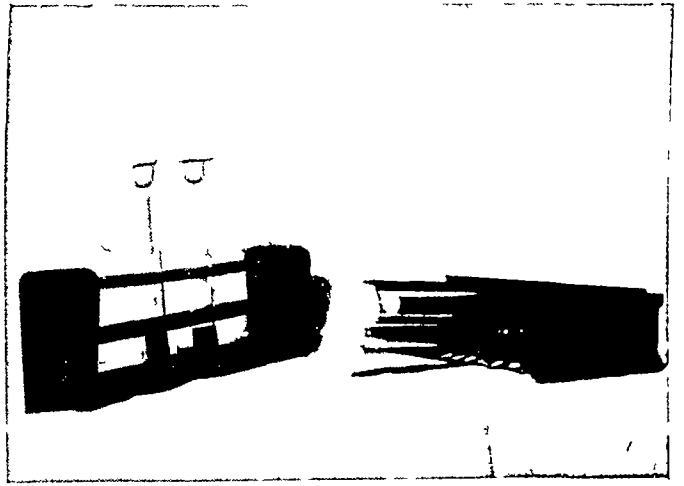


Fig 1 —Test tube stands Note the shape of the sideboard

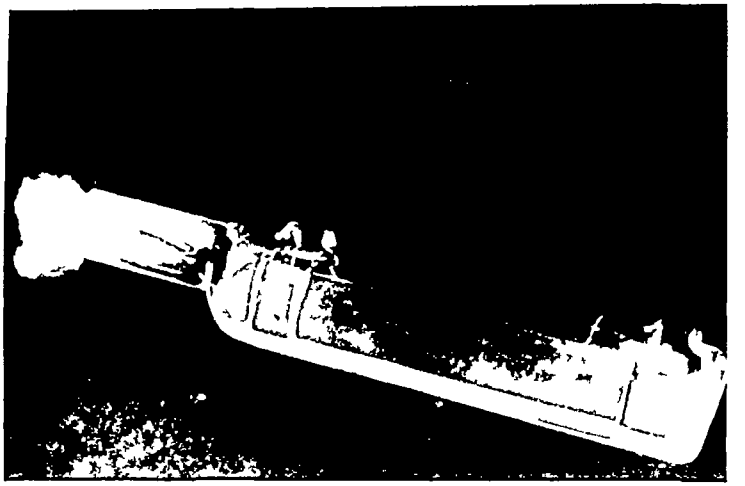


Fig 2 —Legroux tube, large model

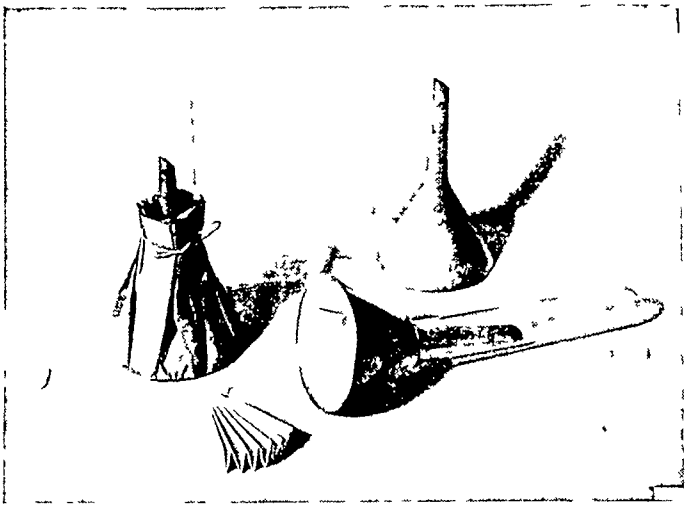


Fig 3 —Assembling the aluminium funnel for filtration through Kiselguhr covered paper

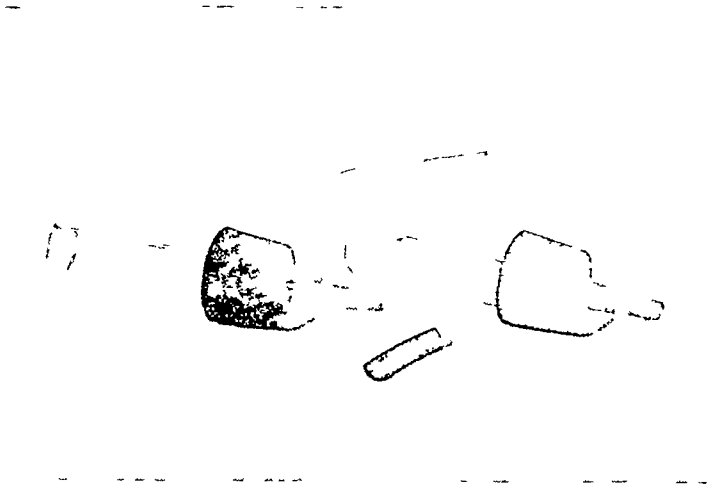


Fig 4 —Cylindrical funnels to be used with L3 candles

Fig 5 —Apparatus for washing big Chamberland candles F or B (right) On the left—two different models of L candles An apparatus for washing them is shown in the Text figure on p 1114

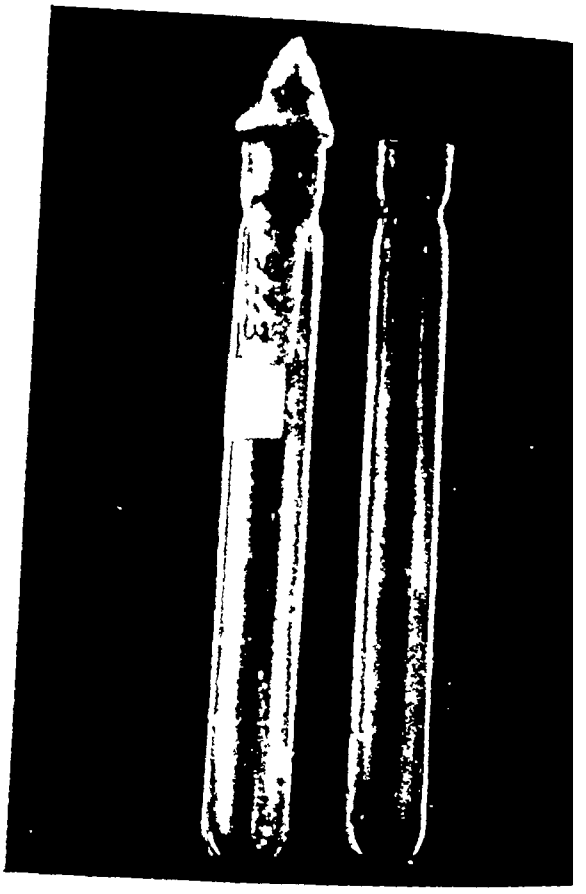


Fig 6 —Constriction tubes, 5″×1″

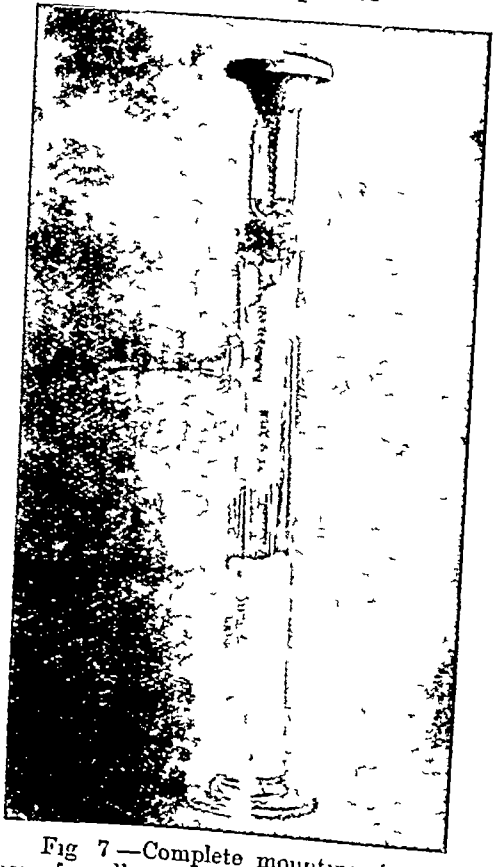


Fig 7 —Complete mounting for filtration of small amounts of liquid Martin flask, aluminium funnel, glass cylindrical funnel L3 candle and constriction tube

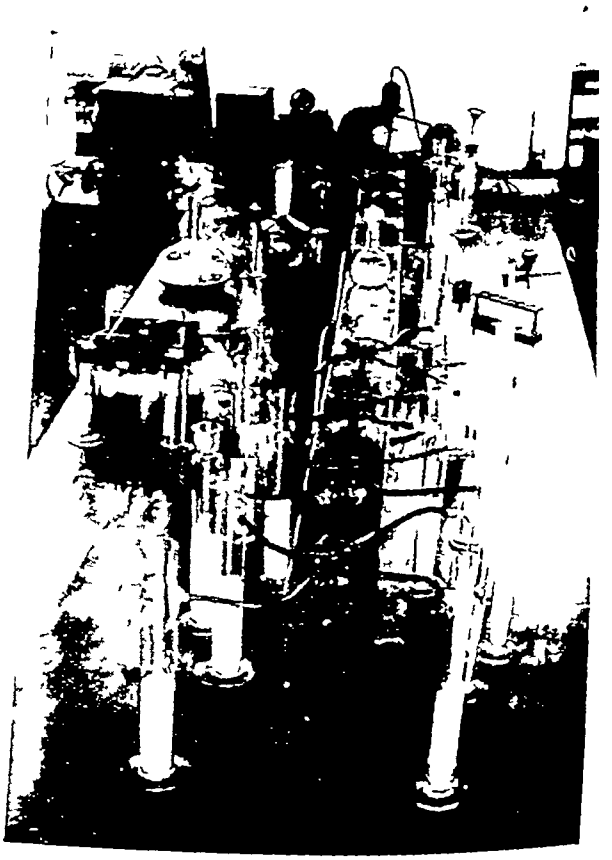


Fig 8 —Filtration table.

By means of the foot-pump the pressure is brought up to 15 lb and then very gradually up to 22–23 lb The pressure at the first appearance of air bubbles is noted If they appear at less than 18 lb the candle must be rejected as defective If they appear only after 22–23 lb the candle is clogged and must be burned and washed again A sound F candle gives the bubbles between 18 and 20 lb

The L3 candles are mounted on so-called *constriction tubes* The French model usually supplied with the filtering outfit is too thin and of inconvenient size either too short or too long The collar part is not satisfactory The 'constriction tubes' of my design, at a price only slightly higher than that of the ordinary test-tubes, were made for me in India* Their dimensions are those of our usual test-tubes $8'' \times 1''$, with a constriction $\frac{3}{4}''$ below the rim These provide a much better hold for the candle The candle must be mounted very tightly by means of non-absorbent cotton-wool, so as to prevent the candle from slipping inside or falling off the tube The opening of the candle is plugged (Plate XLVII, fig 6) The mounted tubes are sterilized in a hot air sterilizer

Sterile cotton-wool plugs, to replace the candle after filtration, are prepared, each wrapped separately in paper

So-called Martin flasks are used for filtration (Plate XLVII, fig 7)

Filtration table —As a considerable number of filtrations is done every day, a special filtration table was constructed (Plate XLVII, fig 8) It is of the usual laboratory type with a shelf running along the middle Under the shelf a gas pipe, with eight quadruple gas cocks on the shelf, is fixed A side hole and channel in each cock converts it into a three-way cock, permitting disconnection of each flask separately The piping is connected to a vaccum tank provided with a gauge The latter is connected to the pump I consider the Crowell Rotary No O-D Vacuum and Blower Pump ('Cenco' Catalogue No 1402) to be a particularly convenient model

Air filter —Often, when admitting the evacuated air back to a container, it is advisable to filter it to prevent contamination Cotton-wool filters are not always satisfactory For such a purpose I have designed the following filter, easily constructed in every laboratory A tall Martin filtration flask is filled almost to the side tube with small glass beads The flask is stoppered with an india-rubber cork through which a glass tube is passed down to the bottom The flask is filled with oil—one inch lower than the surface of the glass beads—lubricating oil discarded from a motor car serves the purpose well The top end of the glass tube is covered with a large pad of cotton-wool The side tubing of the flask is connected to the container where the air is to be admitted The stop-cock or the clamp is slowly

* By the Scientific Instrument Co, Calcutta.

released until the level of the oil in the glass tube starts slowly to fall and then air bubbles will pass through the oil into the container. The principle of this filter is that the air has to pass by intricate ways between the beads through the sticky oil. During that process all the particles suspended in the air, including bacteria, will be retained by the oil (Plate XLVIII, fig. 9).

Collection of material

Stool.—As a considerable number of stools were received by post, a special collecting tube was designed. It consists of a heavy walled test-tube 5"×1" (130 mm × 25 mm), *without rim*, provided with an ordinary cork through which a stout wire (best of aluminium alloy, as it does not rust) as in the usual diphtheria outfit. This tube is protected with a tin etui which, in its turn, fits into a strong wooden box. On the end of the wire a special swab is made: a considerable piece of so-called wood-pulp or lignine is wound round for about three-quarters of the length of the wire. It is then covered with a *very thin* layer of absorbent cotton wool, just to fix the wood-pulp to the wire. A small piece of wood-pulp is placed on the bottom of the tube (Plate XLVIII, fig. 10). As the wood-pulp is about 15 times more absorbent than cotton-wool, such a swab will hold about 6 to 10 c.c. of liquid. The excess will be absorbed by the wood-pulp at the bottom. This arrangement effectively prevents spilling of the infectious material during transit, even if the tube be broken. The wood-pulp can be obtained from laboratory supplying firms (usually gratis as they receive it as packing material). In case of emergency the so-called 'cleansing tissues', used by ladies for removing face cream, can be utilized. To collect the liquid stool it is enough simply to dip the swab into it; it will be absorbed with extreme rapidity. More solid stools can be smeared all over the swab.

However, it was found that much better results for isolation of vibrios were obtained by plating the stool at the bed of the patient direct on Legroux tubes. The technique of such plating is simple and even the subordinate personnel can be easily taught to do it in the field. Nevertheless for isolation of bacteriophage the collecting tubes described above are indispensable.

Water.—River, well or sewage water is best of all, collected into Merck's Peroxide bottles. These bottles stand sterilization well, are very stout and are provided with a good stopper.

Routine procedure of filtration

The sterilized aluminium funnel with the folded paper-filter in it is filled with sterile Kiselguhr suspension. When filtered through, there remains a very thin layer of Kiselguhr on the filter which will retain the majority of bacteria. This funnel is transferred to a candle mounted into a Martin flask and the liquid under

examination is filtered into the cylindrical glass funnel (Plate XLVII, fig 7) However, with heavily laden emulsions of the stool, or when slime-producing bacteria, such as *proteus* or *pyocyaneus* are used, it is advisable to filter the liquid twice through Kieselguhr-covered paper to prevent the clogging of the candle

Suction at 15 inches (100 mm) is applied only when the preliminary filtration has been completed, otherwise the air bubbles in the stem of the glass funnel will prevent the liquid from reaching the candle After the filtration is finished, the aluminium funnel is put into a bucket for sterilization and the glass funnel is disconnected from the candle and put into a saucepan to be boiled The candle is then taken out from the tube cautiously with forceps and also put in water to be boiled The constriction tube with filtrate is plugged with the sterile cotton-wool plug The filtrates are conserved in these tubes and are not transferred to any other receptacles

Routine dilutions and plating of bacteriophage

These serve the purpose of obtaining the discrete clearings produced by bacteriophage in a bacterial layer on agar, proving the presence of bacteriophage for the study of the morphology of the clearings, for isolation of pure-line races of bacteriophage and for enumeration of bacteriophage corpuscles

Requirements —

1 Two 10 c c emulsions of corresponding bacteria, made by addition of about one c c of from 4 to 18 hours old culture to 10 c c of broth, peptone water or tap-water The emulsion must be heavy enough to give, on spreading on the agar surface, a continuous layer of bacteria

2 120×20 mm agar plates

3 A standard 'Small Loop' This is made of 0 3 mm thick platinum or nichrom wire, the inside diameter of the loop being 1 5 mm This loop, if operated as instructed, will take about 0 0015 c c the loop after immersion in the liquid is slowly taken out of it, not flat but sidewise In this case only a thin film will be formed in the opening of the loop, and as its formation depends only on surface tension of the liquid this film will be practically identical every time

4 A standard 'big loop' is made out of 0 5 mm thick platinum or nichrom wire (preferably the latter, which is stronger and more springy), the inside diameter of the loop being 3 mm It is bent in a particular manner (shown in Plate XLVIII, fig 11) to ensure better holding of the drop and comfortable spreading In the case of the big loop, the drop is taken differently The loop is taken out of the liquid flat, with a smooth jerk, when an almost hemispherical drop forms in the loop

(Plate XLVIII, fig 12) One soon learns how to take the right sized drop This drop will contain about 0 025 c c which is the proper amount of the liquid to be spread on a quarter of a 120 mm agar plate The variations in the amount of the liquid here can be considerable, but with a little practice this can be reduced to about 10 per cent, which is not great when dealing with numbers like 10^8–10^{11} In my trials to replace the big loop by Donald's drop method, I found no improvement, while the technique with loops is incomparably simpler

These two loops give us the possibility of obtaining a considerable scale of different dilutions, entirely sufficient for our purposes

The following are the usual dilutions employed —

1 'Small of the small loop' dilution—shortly 'ssl' One small loop of the filtrate is introduced into 10 c c of liquid After thorough shaking, a small loop from this tube is transferred into 10 c c of bacterial emulsion and a big loop of that is plated on a quadrant of the agar plate (Plate XLIX, fig 13)

2 'Small of the big loop' dilution—'sbl' One big loop of the filtrate is introduced into the first tube, a small loop from it into the second, and again a big loop from the last is plated It is easy to understand that the 'big of the small loop' dilution, 'bsl', is identical with 'sbl', the order of the dilution only being changed

3 'Big of the big loop' dilution—'bbl' A big loop of the filtrate into the first tube, a big loop from it into the second and a big loop of the last plated

4 'Small loop' dilution—'sl' Small loop into the second tube, and a big loop plated direct from it

5 'Big loop' dilution—'bl' A big loop of the filtrate into the first tube, and the same amount plated from it

6 When there are very few corpuscles present two more dilutions may be plated 'one c c'—1 c c—of the filtrate into 10 c c of emulsion and a big loop plated from it, and 'direct'—'d'—when a big loop, of the filtrate is spread direct on agar and then covered with one big loop of emulsion

As in all cases it is the same big loop which is plated, this is disregarded in the nomenclature

If we start with the high dilutions, we can plate all seven dilutions using the same two tubes each next dilution containing a considerably larger—about 20 times—number of bacteriophages, the previous one can be disregarded

With the majority of bacteriophage cultures the 'sbl' and 'bbl' dilutions are sufficient to show on one of the platings a sufficient number of well isolated, discrete clearings

As we know the degree of dilution of bacteriophage culture and the amount of the last dilution spread, we can easily calculate the number of corpuscles in our

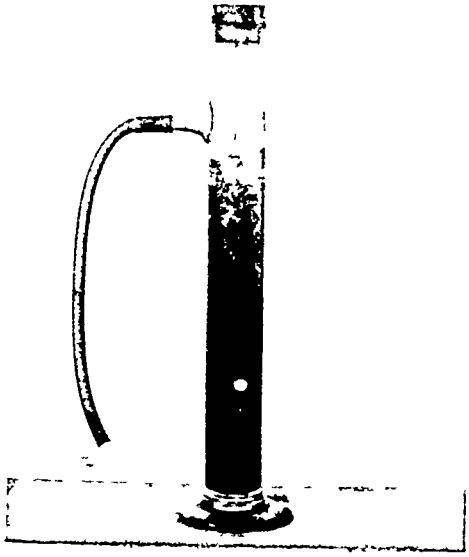


Fig 9 —Air filter

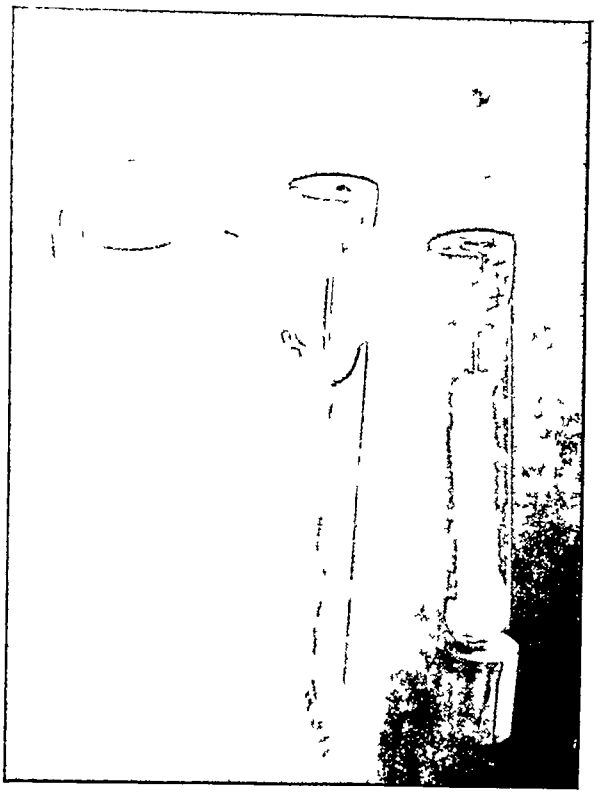


Fig 10 —Stool collection tube.

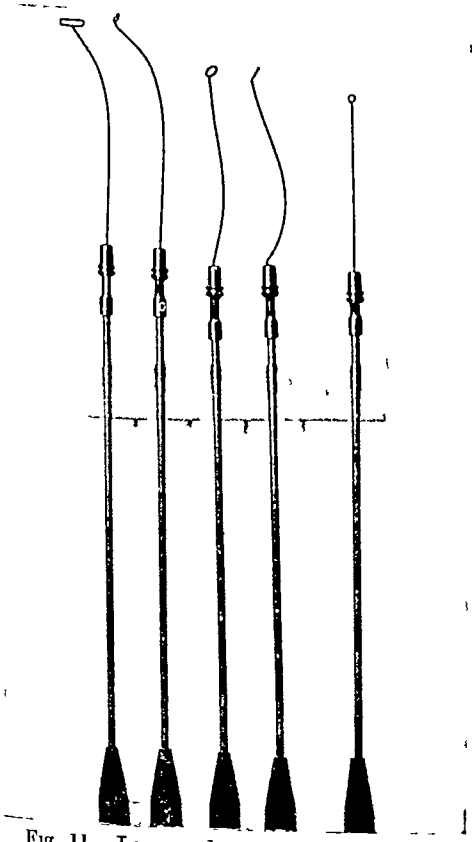


Fig 11 —Loops and spreaders Left to right three quarters and side view of a spreader, three quarters view of 'big loop' Note the peculiar curve which makes it easy to catch the drop and to spread it on agar, a 'small loop'

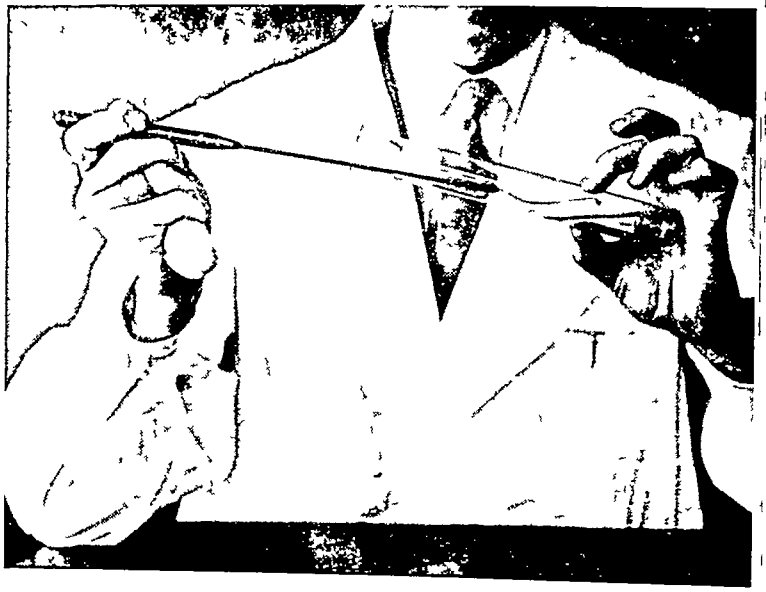


Fig 12 —Taking a 'big loopful'

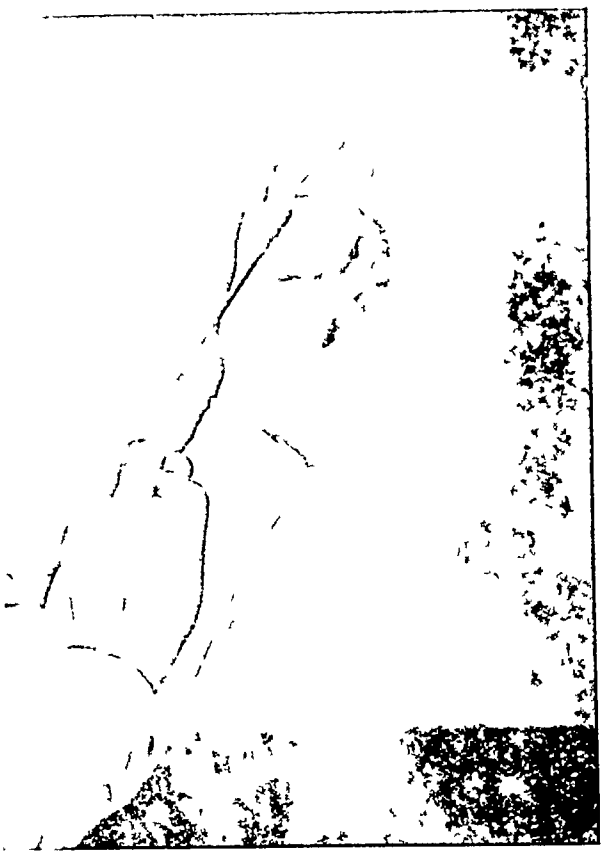


Fig 13 —Plating

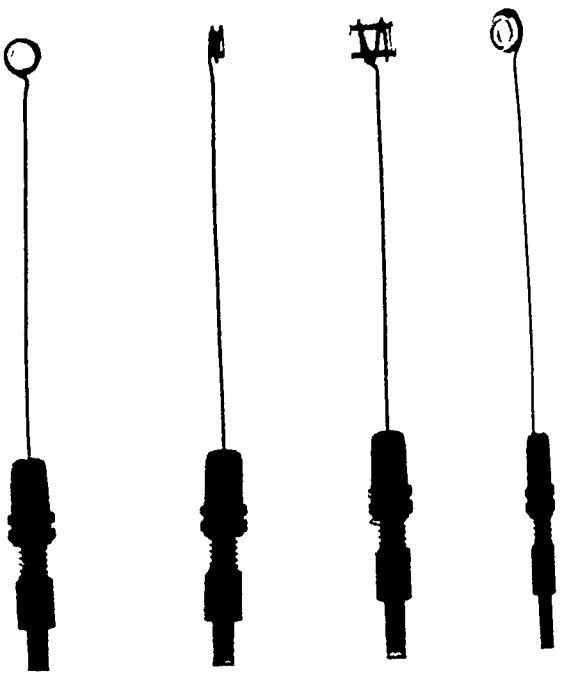


Fig 14 —' Measuring tubes ' The two on the left hold 0 01 c c , the two on the right 0 05 c c

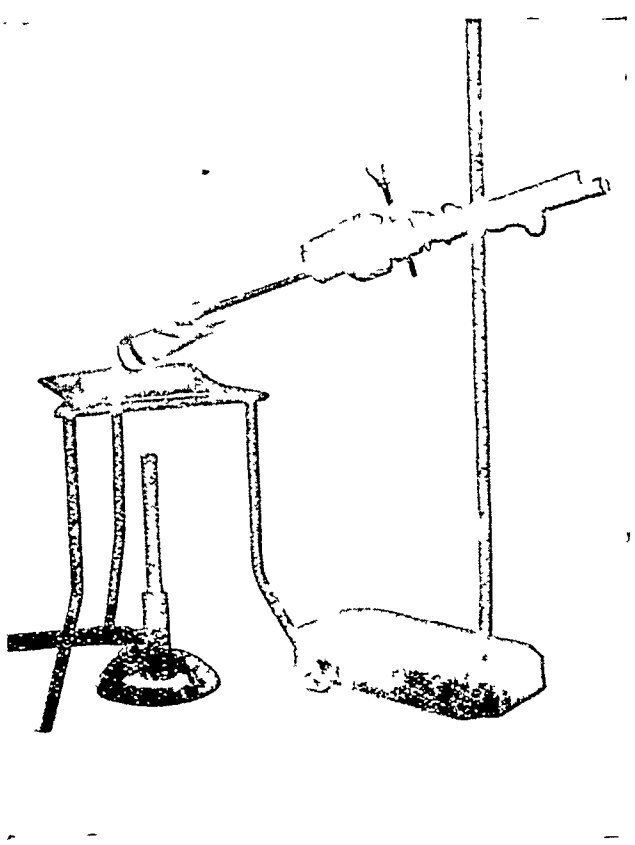


Fig. 15 —Arrangement for sterilization of measuring tubes '

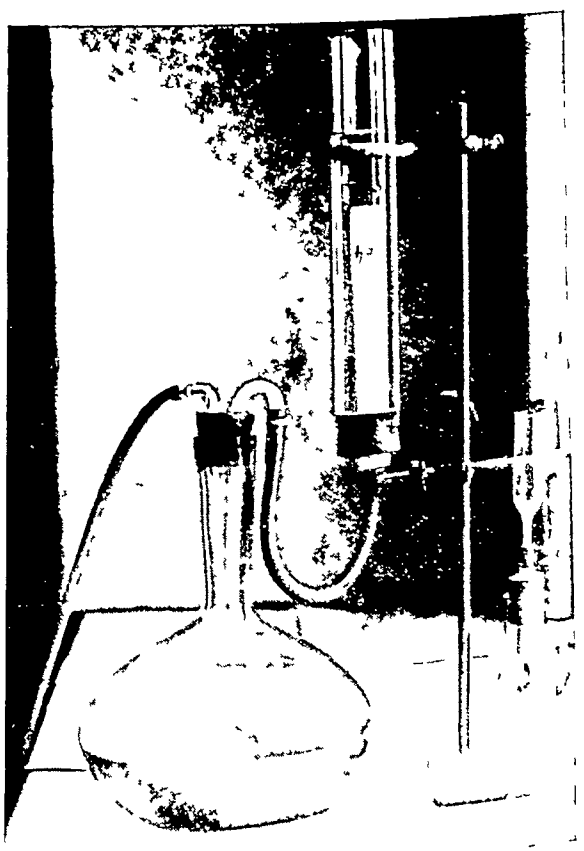


Fig 16 —Arrangement to filter large and small quantities of water for isolation of bacteriophage

original filtrate The following table helps the calculation in case our big loop takes 0 025 (1/10) c c and the small 0 0015 (1/666) c c ,

'bl'	$1\,6\times10^{4}$
'sl'	$2\,6\times10^{5}$
'bbl'	$6\,4\times10^{6}$
'sbl' or 'bsl'	$1\,0\times10^{8}$
'ssl'	$1\,7\times10^{9}$
'1 c c'	$4\,0\times10^{2}$
'd'	$4\,0\times10^{1}$

To use this table multiply the number against the dilution by the number of clearings obtained on the corresponding plating If, for instance, on the 'sbl' plating there are 20 clearings, the filtrate contains $20\times(1\times10^{8})=2\times10^{9}$ corpuscles

The table must of course be calculated anew for each set of new loops, after ascertaining the average amount of liquid taken by these loops This is done by weighing on a sensitive chemical balance To determine the amount of the liquid taken by the small loop, take a small 'agglutination' tube, put about one c c of broth into it and weigh it carefully Then take from the tube ten small loops, drying the loop every time Weigh the tube again Repeat the procedure at least ten times and then calculate the average amount that is taken by one small loop For the big loop either the same procedure is used, except that the weighing is done after one loop is taken, or the loop itself is first weighed dry and then with the liquid taken Not less than ten weighings must be done, or better more, and the average taken

The error of this method can be estimated approximately as 25 per cent, but sometimes it may reach 50 per cent Moreover, the data obtained by different workers can hardly be strictly compared, even if they use the same loops This is not surprising if we take into account the enormous number of corpuscles present in a suspension of bacteriophage and, consequently, the enormous dilutions and insignificant quantity of that suspension used for the actual enumeration of the corpuscles The 'error of chance' in that case is proportional to the degree of dilution

My investigations into evaluation of the virulence of bacteriophage showed the necessity of more exact methods of such enumeration The method of serial dilutions devised by Miquel for counting bacteria, applied by d'Herelle and other workers for bacteriophage work and used as a basis of the technique suggested by Saranjam Khan (1930) and Vardon (1930), gives exaggerated values amounting to from 10 to 1,000 times the actual as verified by plating Therefore it seemed to me that effort must be directed to finding some means enabling us to measure more accurately very small quantities of liquid Donald's (1915) drop method and Wright's pipette method proved to be impracticable in work with bacteriophage

After many trials I succeeded in finding a way of measuring quantities of liquid between 0 01 c c and 0 05 c c with accuracy of about 98 per cent

The method consists in using instead of loops small glass tubes fixed in a wire For instance, to make a tube holding 0 05 c c of the liquid, take a glass tube (preferably Pyrex) of about 3 mm inside diameter and about 8 mm long Run a few spirals of nichrom wire round it, leaving a sufficient length of the wire to fix it in a loop-holder Weigh it on an accurate chemical balance, then dip it into water and take it slowly out Weigh it again If it takes less than 0 05 c c reject it and prepare another from the same tubing, but a little longer If it takes more, cautiously polish the ends of the tube off on a whetstone To obtain a tube taking exactly 0 05 c c —or any fixed amount— is a very tedious job and requires many hours of patient polishing off and weighing However, such an exactitude is not required The important fact is that the quantity of the liquid held by the tube is governed by the law of surface tension and consequently is almost exactly the same every time we take it Therefore, whether our tube will take exactly 0 05 c c or 0 0437 c c is of no importance as it means only that our calculation table will be composed for that particular quantity instead for 0 05 c c , in the same way as we do for loops The small tube, for instance of 0 01 ,c c , is still more difficult to prepare to take a prefixed quantity as it is impossible to cut so narrow a glass ring, and it requires a long time to rub it down on the stone to the required size (Plate XLIX, fig 14)

These tubes cannot be flamed as the loops and are sterilized by boiling For quick smoothly run work I use two ' big tubes ' à 0 05 c c and two ' small tubes ' à 0 01 c c In this case, each pair of tubes must take an equal quantity of liquid, as otherwise the recording of dilutions becomes too complicated To sterilize the tubes two large test-tubes are fixed above a gas burner in an inclined position and filled with well aerated (to prevent explosive boiling) distilled water A bent wire in front of the mouth of the test-tubes helps to hold the loop holders (Plate XLIX, fig 15) I have found that in work with bacteriophage and non-spore-bearing bacteria one minute of the boiling is quite sufficient to prevent contamination, and while one tube is used, there is sufficient time for the other to become ready

The defect of this method is that for the actual plating on agar it is still necessary to use our ordinary ' big loop ', which increases the error to about 10 per cent even in the hands of an experienced worker

I give here, as an example, the calculation table for ' big tube ' of 0 05 c c , ' small tube ' of 0 01 c c , provided that for plating a ' big loop ' of 0 025 c c is used,

' sst '	4×10^{7}
' bst ' or ' sbt '	8×10^{6}
' bbt '	$1\,6\times10^{6}$
' st '	4×10^{4}
' bt '	8×10^{3}

The table is used in the same way as that for loops

I would like to make it quite clear, that in my opinion, using bacteriophage action on bacteria (lysis or clearings) as the criterium of its presence, we will never be able to ascertain the *actual* number of bacteriophage corpuscles present Experience shows that the number of clearings, produced by the same amount of the same bacteriophage suspension, varies with different strains of bacteria used For instance, if we take a suspension of a bacteriophage which has been for a long time maintained in the laboratory on laboratory ' strains of bacteria and plate the same dilution of it, using on the one hand a ' laboratory ' strain of bacteria, and on the other a freshly isolated strain, we will have larger number of clearings with the former On the contrary, a freshly isolated race of bacteriophage will give a larger number of clearings with a freshly isolated strain of bacteria Still more difference will be obtained if we use for plating smooth and rough cultures The difference in this case can be very considerable As each culture varies as to the relative number of rough and smooth elements present it follows that the number of clearings obtained with the same suspension of bacteriophage may vary also Therefore when counting the number of corpuscles, the most we can say is that a suspension of bacteriophage contains so many corpuscles 'active for the particular culture used '

Isolation of bacteriophage

From stool —The standard papain broth is distributed into 200 c c flasks by 50 c c amounts The stool-impregnated swab is soaked in that broth, and then is easily removed from the wire with the help of forceps If the stool is brought to the laboratory in some other container, about 5 c c of the liquid stool or a piece of about the size of a large hazel-nut of the solid is thoroughly mixed with the broth A few drops of a young freshly isolated peptone water* culture of the organism against which the bacteriophage is sought is added The addition of the fresh culture aims at giving the bacteriophage probably present the opportunity to develop even during this first cultivation and to be consequently more easily discoverable I have used this enrichment method with success since 1923 (Asheshov, 1924) The flask is left till next day, when it is filtered and the filtrate examined for the presence of bacteriophage During cultivation the reaction is corrected if necessary

From water —The use of the enrichment method is essential Water, and particularly sewage water, contains a large amount of bacteria, forming on the surface of the broth a pellicle which intercepts the free supply of air to the rest of the broth, impeding in that way the normal development of bacteriophage

* For work with dysentery bacteriophage d'Herelle recommends preparing bacterial emulsion from agar slope cultures in preference to the broth cultures We have found that for cholera work young—6 to 10 hours old—peptone water culture gives better results than washings from agar slopes

Therefore it was found essential to filter water before adding it to the broth It is advisable to use a large amount of water, from 100 to 2,000 c c The following technique was developed —

Into the Kitasato filtering flasks of necessary size a certain amount of concentrated papain broth, or 20 per cent peptone water is added The amount is calculated by the amount of water which is to be filtered into this flask, so that the final content of the liquid in the flask in nutrient material will be approximately the standard one of '0 5 per cent oxidizable matter' content, or 1 per cent peptone Phenol red solution is added calculated for the total amount of the resulting liquid If a flask of not more than 500 c c is used, it is mounted with L3 candle, 75×15 mm or 150×15 mm , as shown in Plate XLIX, fig 16, and the whole sterilized Flasks of one or two litres are better mounted with larger candles I do not advise filtering more than 200 c c into a 500 c c flask, or 800 to 1,000 c c into a 2,000 c c flask, as the essential supply of air will not be sufficient For larger amounts of water, I would advise using the big Pyrex balloons used for preparation of vaccine They are mounted in the same way as big Kitasato flasks (Plate XLIX, fig 16) After filtration the india-rubber cork is replaced with a cotton-wool plug As enrichment here is essential, a young, freshly isolated culture is added, one to two c c for every hundred c c of the liquid, and the flask is left till next day The reaction, if necessary, is corrected during cultivation by addition of sterile 4 per cent NaOH solution

Next day some 20 c c from the flask is filtered, dilutions in ordinary broth are prepared and platings of 'bbl', 'sl', 'bl' and '1 c c' are made After that, 5 c c of the filtrate is added to the first tube, 2 5 c c from it transferred into the second and all are put into an incubator in a slanting position A control tube with the same amount of culture is placed along with them The tubes during the first 6 to 10 hours are observed every ½ or 1 hour, and the change in the tubes as to the turbidity and reaction of the medium is noticed

The following conditions may be present —

1 During all the period of observation, and the next day the tubes present no difference from the control, and the bacterial layer on the platings is normal This absence of bacteriophage may be only apparent, as has been ascertained many times Therefore both inoculated tubes are mixed, filtered and the procedure of plating is repeated in the same manner If after the second transfer there is no trace of lysis and the bacterial layer remains normal, bacteriophage is considered absent

2 The inoculated tubes become at some time during the cultivation less turbid than the control, though the next day this difference may disappear, or the inoculated tubes may even become more turbid than the control The platings may show either few clearings or only 'traces' of them, that is to say round spots, perhaps even of pinhole size, entirely covered with bacterial growth, but of

different character from the surrounding layer, or the whole layer is somewhat different from the control it can be more transparent or more opaque, or granular This may indicate the presence of bacteriophage though a feeble one In that case the tubes are filtered and ' bl ' and ' 1 c c ' platings are made If no definite clearings are obtained, but it is still of interest to work with this feeble bacteriophage, trials can be made to augment its virulence as described later

3 There is definite lysis at some time during the cultivation, which can later on be masked by the appearance of secondary growth or may remain more or less permanent Plating presents anything from isolated well defined clearings to isolated colonies of resistant growth, or even a sterile surface In that case an active bacteriophage is present The tubes are filtered and ' sbl ' and ' bbl ' platings are made for the study of the morphology of the clearings and isolation of pure line cultures

Short-cut method of ascertaining the presence of bacteriophage—This gives positive results when bacteriophage is present in relatively large number, at least 5×10^2 per 1 c c On the surface of the agar in Petri dish or in Legroux tube, or even on a agar slant an emulsion of corresponding bacteria is spread so as to obtain a continuous layer of growth When the surface is dry, a drop of the filtrate is put over it If bacteriophage is present, next day we shall see a big clearing present in the layer of bacteria Farther on in this paper another short-cut method will be described which at the same time will give the indications of the types of bacteriophage present in our filtrate

Isolation of pure-line types

After the presence of bacteriophage is confirmed, and the well-isolated discrete clearings are obtained, then morphology must be attentively studied It is necessary to do this after as few transfers as possible Quite often there can be present two bacteriophages of widely different rates of multiplication, and consequent transfers can eliminate the ' slow ' one, or reduce it to such a small number that on the usual platings there will be visible only one type Therefore it is advisable sometimes, besides making dilutions giving discrete clearings, to make platings with a large amount of filtrate like ' bl ' If another bacteriophage is present in small numbers its presence, perhaps, will be discovered by clearings, or traces of clearings, in the layer of secondary growth resistant to the predominating type For the same reason, if one does not want to lose a bacteriophage possibly present along with the other but in small number, it is better to keep the original filtrate and then examine it by means of the type test described below If bacteriophage is present, but the ordinary method of isolation from single clearing is not successful, this can be done by means of anti-serum as will be described in a further publication

The character of the clearings must be studied The size alone is in the majority of cases of no importance Bacteriophages are met with, particularly amongst the dysenteryphages which are without any doubt pure cultures of one type but giving constantly clearings of a size varying from many millimetres to pin-point It is the morphology, the structure of the clearing which is important The character of the centre, the inner edge of the phage zone, the character of that zone, its outer edge, the lysin zone, the character of the secondary growth appearing in the clearing—all must be noted But usually, after a very little practice, the presence of two types of clearings together on the same plating is recognized at a glance It gives a somewhat 'patchy' impression A closer study will reveal the difference

If the presence of different clearings is noticed, they are separated on the same principle as bacteria cultures are isolated For each type two tubes with emulsion of corresponding bacteria of about 100 to 150 millions per c c are prepared For isolation a very small loop, made of very thin wire, is used its 'light' must be of pin hole size, the flat part slightly bent With the flat of this loop the centre of a well-isolated clearing is repeatedly dabbed A slight amount of liquid, which in that manner is pressed out from the agar, helps bacteriophage to adhere to the loop The loop is washed off in the emulsion and the following four platings are made (1) direct—'d'—plating from the first tube, (2) a 'small loop'—'sl'—from the first tube is transferred into a second, also containing emulsion, wherefrom the usual plating is made, and (3) in the same manner a 'big loop'—'bl'—is transferred and plated One of these platings is sure to give discrete clearings The 'd' plating moreover frequently helps one to recognize if the isolation has not been successful and another type is still present in small numbers If necessary the process of isolation is repeated In any case, after one or two passages by means of filtration the control platings should be made, including that of a large quantity, say, 'bl' The practice of this laboratory, and which is strongly recommended, is to plate after each passage It saves the disappointment of unexpected results and adds much to the exactness of observations, giving often very important information as to the state of the bacteriophage culture Exact methods of control of bacteriophage cultures for their purity will be given further on in the paper, under the discussion of different types of bacteriophage

Here it will be not out of place to mention the necessary precautions to protect bacteriophage cultures from contaminations Some of them will perhaps sound elementary Unfortunately, in many laboratories they nevertheless are not observed

1 Prevent bacteriophage from being spread all over the laboratory, treating each filtrate as a highly contagious liquid If spilt, use freely some chlorine solution, electrolytic chlorine (E C) for instance This solution must be in hand's reach of every worker Hands must be frequently washed in it

2 Have a piece of cotton-wool moistened in E C on a Petri dish cover in front of you when transferring or plating bacteriophage Drain the loop on it before flaming the droplets of the liquid brought suddenly into the flame burst and scatter bacteriophage all round before it is killed in the flame

3 When pouring out bacteriophage suspensions from any container (e g , from a test-tube into the funnel), flame the rim of it not only before but after pouring out as well The container before sterilization is likely to be handled by attendants, who will soil their hands and spread bacteriophage all over the laboratory

4 Flame the rim of the filter candle before inserting the stopper of the funnel When disconnecting filter candles, wipe the place of connection with cotton-wool moistened in E C before touching the candle

Summary

The technique of work with bacteriophage devised mainly for investigations on choleraphage, but applicable in research on any bacteriophage, is described

The basic principle of this technique is to treat bacteriophage as an ultramicroscopic, 'filtrable' living organism The successful use of this technique during many years is considered as an additional proof of the living nature of bacteriophage

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STUDIES ON CHOLERA BACTERIOPHAGE

Part II

CLASSIFICATION OF BACTERIOPHAGE AND ITS PRACTICAL APPLICATION

BY

IGOR N ASHESHOV, INNA ASHESHOV, SARANJAM KHAN, M N LAHIRI AND S K CHATTERJI

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I HAVE already pointed out (Asheshov, 1924) the existence of bacteriophages producing different kinds of clearings and the possibility of separating them in pure-line cultures, breeding true (Plate L, figs 1 and 2) Before that other authors also paid attention to the difference in formation of clearings, but their differentiation was mainly concerned with the size of the clearings, and not their structure

MORPHOLOGY OF THE CLEARINGS

For the study of morphology of the clearings we can distinguish the following parts in them and their variations (*see* Plate L, figs 3 and 4, and Plate LI, figs 5 and 6) —

1 *The clearing proper* —This is the central part of the clearing which can be free from bacterial growth, or contain isolated colonies of resistant bacteria, or be

covered with a bacterial layer The character of this growth is noted Even if the growth covers the clearing proper completely, it usually presents a definite difference in appearance from the surrounding normal growth of bacteria In examining the clearing proper we notice its size (which is least important), the character of its edge, and the character of secondary culture present in it Usually we describe the edge of the clearing proper only in cases where it is not surrounded by zones In that case it is usually abrupt,—almost perpendicular to the surface of the agar If the clearing proper is surrounded by zones we prefer to describe that edge as the inner edge of the zone as actually it is a part of it

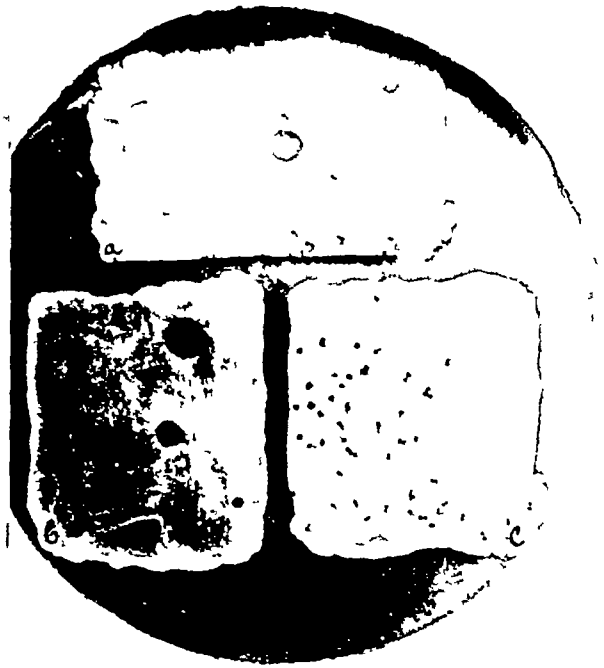
2 *Bacteriophage zone*.—Here we note the size it can be a very thin ring, only a fraction of a millimetre wide, or may reach many millimetres The character of the inner edge it can be smooth or indentated, sloping gradually or abruptly, of the same structure as the zone or different, presenting itself as, e g, a whitish ring The outer edge abrupt or sloping sharply outlined or gradually diffusing, circular or indentated

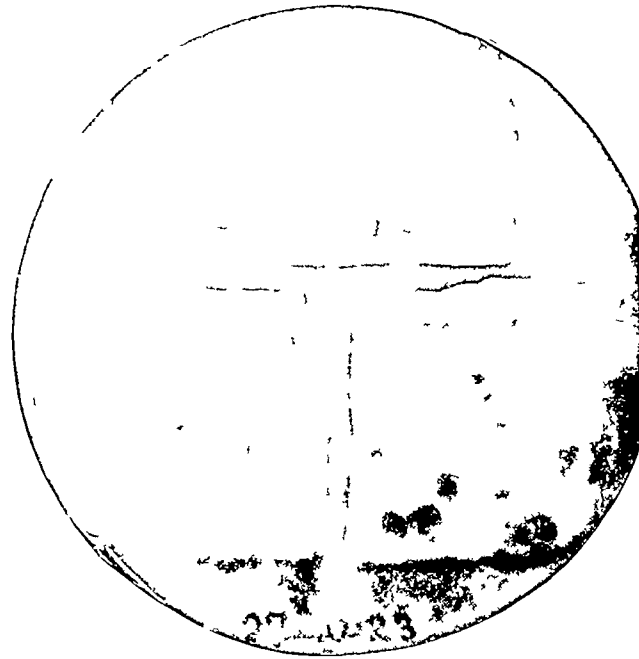
This zone contains bacteriophage on its whole surface,—hence the name It is probably the result of penetration of bacteriophage into the bacterial layer at the time when the latter could still be partly influenced by the former

3 *The outer or lytic zone*.—The same parts as in the phage zone are noted It is of interest to study the rapidity and intensity of penetration of this zone into the normal layer of bacteria surrounding it Some bacteriophages evidently produce a large amount of powerful lysin, which rapidly diffuses into the bacterial layer, changing within a short time its whole available surface A good example of this is given by Sertić (1929) which enabled him to prove definitely the production of lysins by bacteriophage He also studied the penetration of bacteriophage into that zone Here bacteriophage itself evidently penetrates the bacterial layer when it cannot any longer produce any visible changes in it, as its presence or absence in different parts of this zone makes no difference as to the appearance of the latter

As I have mentioned above, the size of the clearings is of no great importance It is true that some bacteriophages usually produce large clearings, some small But with the majority of bacteriophages this characteristic is not constant and can be modified at will to a considerable extent, particularly in diminishing the size by repeated selection of the smallest clearings As we will see later, bacteriophage belonging to the same type can produce clearings of very different size

Though the morphology of the clearings is a very important characteristic of bacteriophage, we must never forget that it is not a permanent one and depends on the environment—in fact to the same extent as in the case of morphology of bacterial colonies Therefore the study of morphology of the clearings has only a comparative value, assisting us in the comparative study of different types of bacteriophage





Figs 1 and 2 Mixed and separated in pure line cultures of dysenteryphages

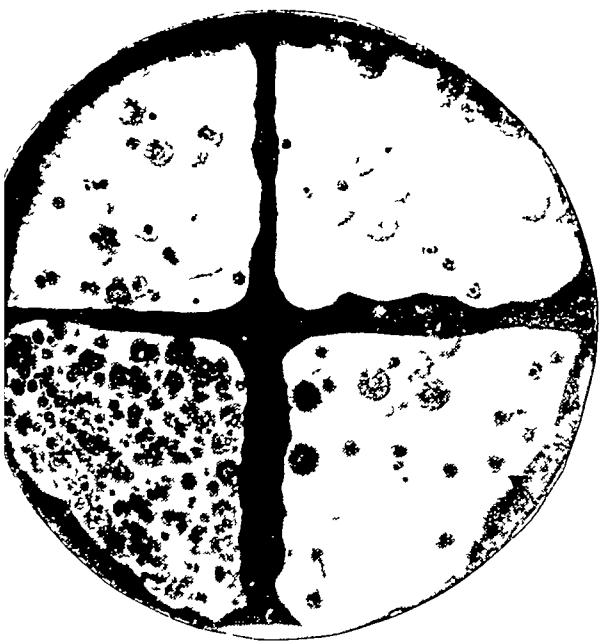


Fig 3 Different types of coli phages, showing morphological varieties

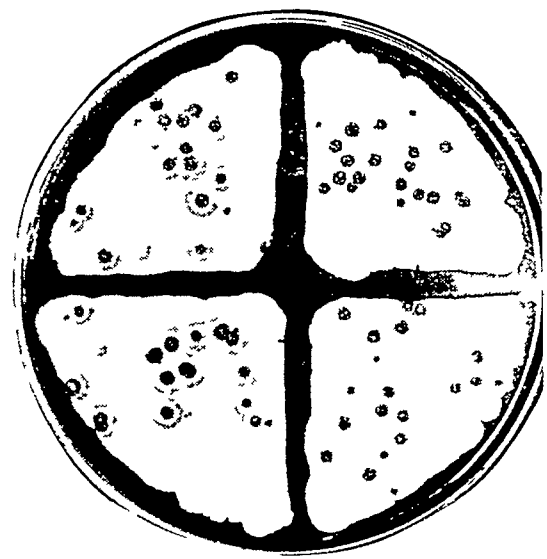


Fig 4 Two types of coli phage, one showing large lytic zone

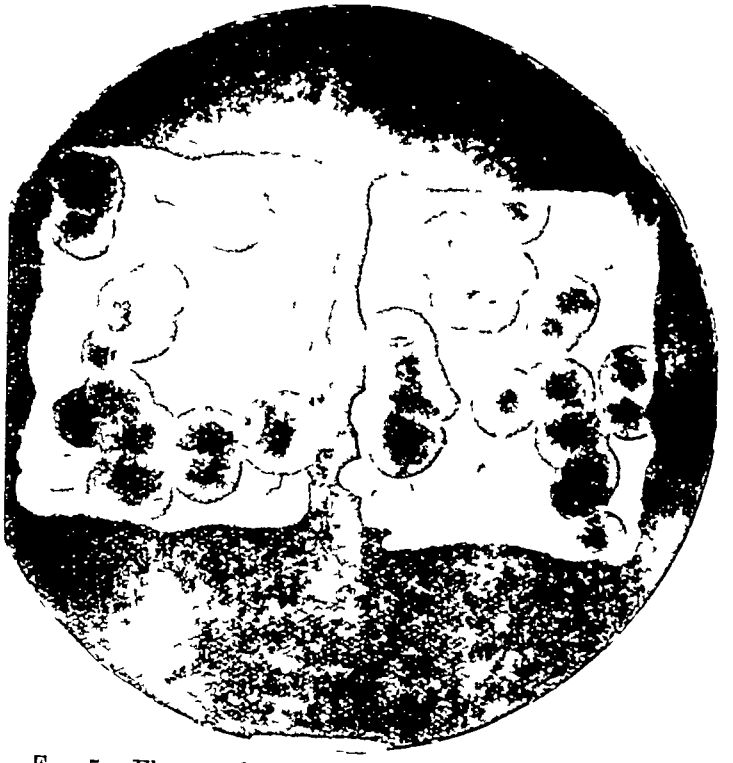


Fig. 5 Flexner-phage showing large bacteriophage zone No lytic zone

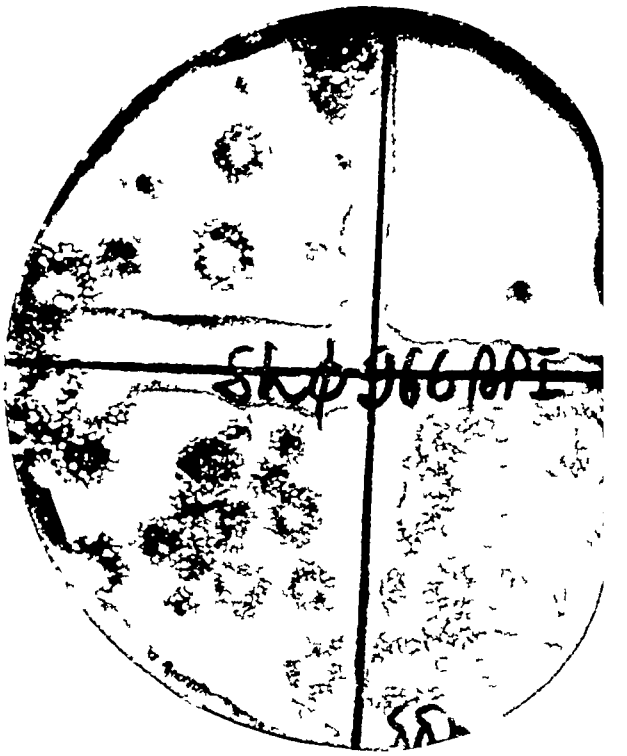
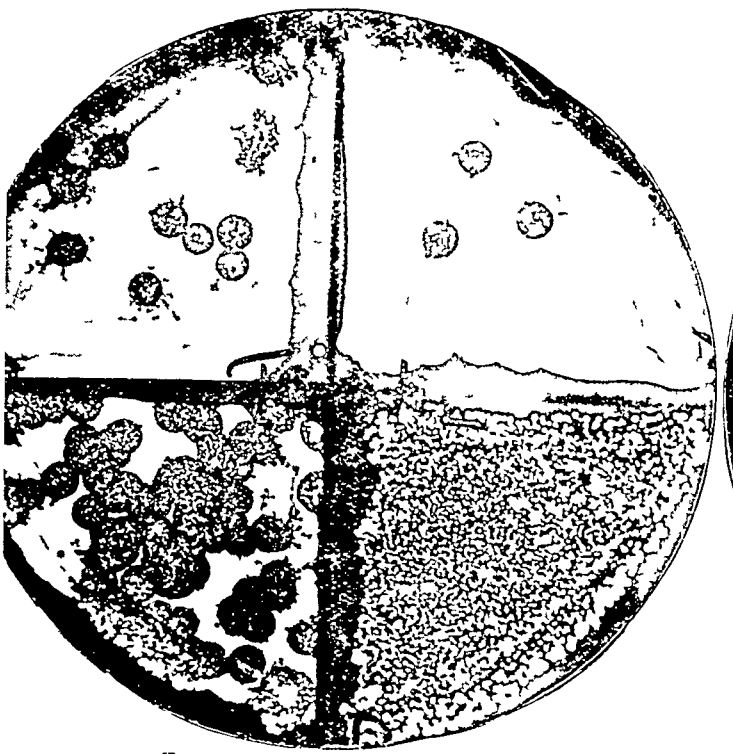
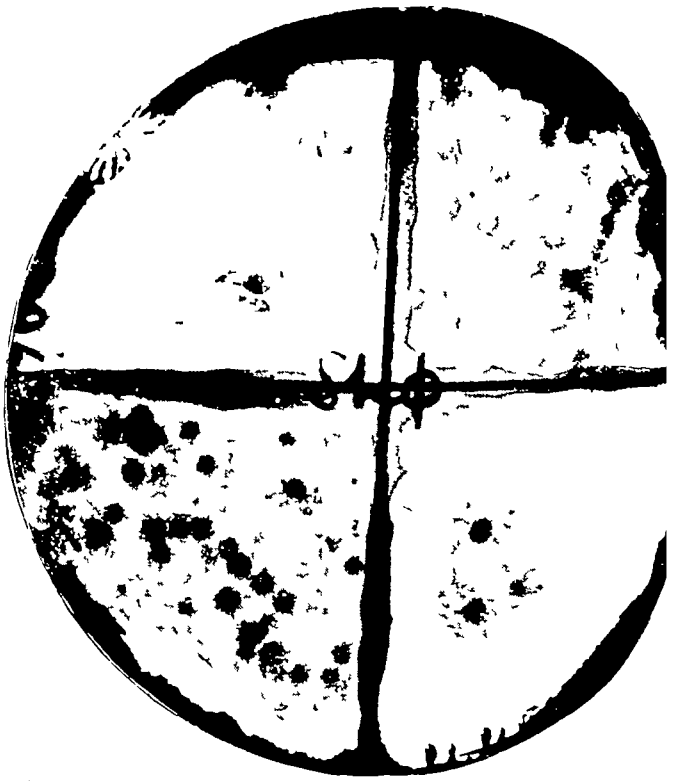


Fig 6 Shiga phage with well defined bacteriophage zone, lytic zone hardly visible





Figs. 7 and 8 Peculiar forms of clearings of dysenteryphages, sometimes met on slightly wet agar.

The morphology of the clearings depends on the following factors —

1 *Bacterial culture* —The character of the culture and its state influences the formation of the clearings directly any alteration in it is followed by a change in the character of the clearings On different strains of the same bacteria a bacteriophage can produce entirely different clearings Even if we take a bacterial culture and by plating it on agar obtain a dozen strains picked from single colonies, we may get a different type of clearing with each strain It follows that any change in the relative amount of these component parts of the culture will change the aspect of the clearings of the same bacteriophage So, for instance, the phase of dissociation of the culture influences the formation of the clearings considerably

2 *The nutrient medium* —This influences the formation of the clearings by its chemical composition and by its physical state Generally speaking the chemical composition influences it indirectly by altering the character of the culture Bacteriophage is very sensitive to these alterations Even the usual slight differences in composition of different batches of the same agar alter the aspect of the clearings, and this is the main difficulty in the study of their morphology The only method which would permit us to obtain more constant results would be the use of a medium of definite chemical composition For *B coli* we have the excellent synthetic medium of Sertić (*loc cit*) which brings out the structure of the clearings particularly well (Plate L, fig 3) Here is its composition —

Asparagin	5 g
Magnesium sulph	1 ,,
Potass sulph	1 ,,
Sodium monophosphate	2 ,,
Glucose	1 5 g
Lactose	0 5 g

Reduce the asparagin to powder and dissolve it in some 100 c c of hot distilled water Dissolve the other ingredients each separately in a small quantity of water Add the solution of phosphate to the asparagin solution, mix the two sulphates and add them to the asparagin and phosphate, then add the sugars Complete to 1,000 c c with distilled water This is the only way to obtain a clear solution, the reaction of which is pH 7 2 which on addition of 1 1 per cent of agar and sterilization will be strictly neutral Bacto-Agar Standardized is used Do not clear with egg

Unfortunately this medium cannot be used for the study of choleraphage as cholera vibrios grow very poorly on it I have tried many combinations with a view to obtaining a synthetic medium for cholera vibrio but without success Not one of them gave sufficiently good growth of the vibrio to show well the morphology of clearings

The physical state of the medium, or to be more precise, its consistency, influences the formation of clearings in two ways (*a*) through influence on the bacterial growth, and (*b*) directly on the bacteriophage The greater the concentration of agar-agar in the medium, the less permeable it is The results of lesser permeability is that, on the one hand the nutrient ingredients reach the bacteria with more difficulty, while on the other hand the products of metabolism of both bacteria and bacteriophage do not diffuse away from the point of growth but accumulate and interfere with further development of bacteria and bacteriophage That is why the concentration of agar must be as low as possible As I have pointed out before we use as routine 1 1 per cent agar

On account of this instability in the morphological character of the clearings, we cannot take it as the basis for classification of bacteriophage On the other hand the morphological differences of bacteriophage races are of considerable practical importance They give us a practical method of separation of pure-line races, because, however great may be the variations in morphology of the clearings of one and the same race of bacteriophage under the influence of environment, different types of bacteriophage will almost always preserve under any circumstances the difference between themselves and we can always distinguish them one from another For instance, if we plate two types of bacteriophage together, in the majority of cases we will be able to distinguish them by the morphology of the clearings and to separate them, though we probably will not be able to recognize which is which, owing to unknown changes introduced by the environment

Very early in my studies I observed one more phenomenon which makes the use of morphological characteristics for differentiation of bacteriophage still less certain This phenomenon (also partly described by Sertić) in my opinion closely resembles the phenomenon of dissociation in bacteria though being entirely independent from it Occasionally, under influences as yet unknown, some races of bacteriophage begin to produce two kinds of clearings So, for instance, in cholera-phage a race of Type A, which usually gives zoneless clearings, will start to produce on agar, besides the typical clearings, clearings with zone, which resemble (but are not identical with) clearings of choleraphage Type B Or choleraphage Type B will give, besides the usual clearings, clearings with a well-pronounced white ring on the inner part of the first zone (Plate LII, figs 9 to 11) Isolated in the usual way, these two kinds again imitate the process of dissociation one kind breeds almost true, the other continues to give two kinds of clearings in spite of very many isolations from a single clearing At the same time the other 'type characteristics', which will be described later, remain unaltered, both kinds of bacteriophage remaining true to the type from every other point of view

If the transfers are continued without isolation, the newly appearing kind sometimes overgrows and suppresses the original More often we observe this phenomenon with the newly isolated races of bacteriophage. My impression is

that this is due to the new environment induced by our artificial methods of cultivation, vastly different from those which bacteriophage meets in nature In every report to the Conference of Research Workers in India I have insisted on the fact that by using our laboratory methods of cultivation of bacteriophage we induce considerable changes in bacteriophage cultures,—' denaturing ' them—and that bacteriophage translated from its natural conditions and placed in artificial ones, such as a test-tube with broth, loses very quickly many of its valuable properties and acquires the new ones not possessed by the ' natural ' bacteriophage

Reciprocal compensatory action of different types of bacteriophage

The morphological differences in the clearings produced by bacteriophage impressed on me the necessity of dividing them into definite groups or types to classify them Many authors noted that there exist bacteriophages possessing different properties, but no definite attempt at classification, at systematizing our knowledge of bacteriophage, was made Exception must be made only in the case of Burnet (1929) who was the first to suggest the division of bacteriophages into three groups (1) bacteriophage acting only on smooth elements of the culture (' smooth ' phage), (2) bacteriophage acting on rough elements only (' rough ' phage) and (3) bacteriophage acting on both smooth and rough elements (' smooth-rough ' phage) We verified the observations of Burnet and were able to confirm his statements

In the following discussion I will refer mainly to our observations on cholera bacteriophages, which give a good example for classification Experience proves that the same principles can be applied to other bacteriophages as well

Examining at the beginning of my work on choleraphage many hundreds of races of choleraphage I was able, at first only on the basis of the morphological character of the clearings, to separate them into three groups the races of choleraphage of each group gave clearings of the same kind different from those produced by bacteriophages belonging to the other groups To these groups I gave the name of Types A, B and C For convenience we use the following symbols for them ChϕA, ChϕB and ChϕC (Plate LII, fig 12)

The question arose whether this division would be found to be supported by other properties peculiar to each type so separated Experiments show the following —

1 The difference in morphology of the clearings coincided with Burnet's division ChϕA acted only on smooth elements, ChϕB on both smooth and rough, ChϕC almost exclusively on rough However, though the division of bacteriophages into ' smooth ', ' smooth-rough ' and ' rough ' is very important, it is not

always practicable, particularly in the case of bacteriophages acting mainly on rough elements. Every culture, even the 'smoothest', especially that of cholera vibrios, contains some rough elements, on which even a strictly 'rough' bacteriophage can propagate itself. Besides, as we shall see, this division proves to be insufficient, as each group may contain bacteriophages of considerably different character. For instance ChϕB, ChϕC and ChϕD (recently described by Pasricha) differ widely in their properties one from another, though all of them belong to the 'smooth-rough' bacteriophages. Therefore I prefer to use the property of a bacteriophage of acting in preference on the certain phase of dissociation of a bacterial culture not as a criterium for classification but as one of the permanent characteristics of the type. But understanding of the existence of such a property is essential as it explains many phenomena which otherwise could bring confusion into our principles of classification.

As an example of such a phenomenon I will cite one of Bail's (1923, Table on p. 134) experiments, which he found difficult to explain. He possessed a bacteriophage 'Yk' which did not show an evident action on a bacillus called 'Kraus'. However, when 'Kraus' was submitted to the action of another bacteriophage 'Km' and a secondary growth of 'Kraus' resistant to 'Km' was obtained, the latter showing itself sensitive to the action of bacteriophage 'Yk'. My explanation of this paradox is that bacteriophages 'Yk' and 'Km' belonged to different types and to different groups of Burnet's division. 'Yk' may be, for instance, a strictly 'rough' bacteriophage. Then bacillus 'Kraus' must have been smooth with only very small number of rough elements present. Therefore the action on it of 'Yk' could not be ascertained by the method of plating. Bacteriophage 'Km' then was a strictly 'smooth' one. Its action destroyed all smooth elements of 'Kraus', leaving the rough untouched, which formed the 'Km-fast' growth. Being rough, this culture easily showed the action of bacteriophage 'Yk'. A similar example we find in the recently published paper by Pasricha (1932) on ChϕE. A culture of cholera vibrio which seemed to be naturally resistant to this bacteriophage became sensitive to it after the action of ChϕA. The explanation here is exactly the same as in case of Bail's bacteriophages 'Yk' and 'Km'.

2. Antigenically my three types of choleraphage (those discovered by Pasricha are not yet investigated) are unmistakably different: anti-ChϕA serum inhibits only the action of ChϕA, leaving intact all the others, ChϕB inhibits only ChϕB, etc. This will be dealt with *in extenso* in a special paper.

3. Most important from the practical as well as the theoretical point of view is the fact that vibrios which became resistant to the action of one type of bacteriophage are still sensitive to the action of all the remaining types. In other words, the secondary growth of vibrios appearing after the action of, say, ChϕA and resistant to it, is still sensitive to and can be destroyed by the action of all

bacteriophages belonging to other types This is reciprocal to all the types, as the following experiment proves —

Five tubes of the usual emulsion of vibrios in broth are inoculated, each tube separately, with pure-line cultures of the five types of choleraphage After complete or partial lysis in these tubes the secondary resistant growth will appear Each growth is spread separately in columns on the surface of agar in a Petri dish Filter all the tubes Superimpose on each of the spreadings one drop of each of the filtrates which contain the pure-line cultures of choleraphage When the bacterial layer on the agar becomes visible, we will see that on the secondary growth resistant to ChϕA all the other bacteriophages have produced clearings, similarly the secondary growth resistant to ChϕB will be found resistant to ChϕB only, and so on (Plate LII, fig 13)

These three characteristics of the types of bacteriophage are stable and permanent we have never yet seen a bacteriophage belonging to one type lose one of these properties or acquire one peculiar to another type Neither have we met yet an intermediate type This statement worked out first for the three types of choleraphage was entirely supported by the ChϕD and ChϕE recently described by Pasricha (1932) Therefore I consider the suggested division not only logical and natural but also indispensable for the rational study of bacteriophage The fact that we were able to apply with the same success the same principle of division and classification to dysenteryphages and some other intestinal bacteriophages still further supports our view

The fact that the secondary culture which has become resistant to one bacteriophage may still be sensitive to another has long been known Bail (1923) in particular studied this phenomenon to a considerable extent Other workers confirmed his observations, but they were not applied to work out a definite method and were not used for a definite classification of bacteriophages which alone can bring a clear system into our studies

Properties of different types of choleraphage

Besides the three cardinal and permanent characteristics mentioned, bacteriophages belonging to the same types have many other properties common to them, though not permanent and varying from race to race under the influence of the environment The following is the more detailed description of the types of choleraphages described up to now —

ChϕA is a quick acting bacteriophage the best race of this type can produce a complete lysis of vibrios in less than two hours Its generation period is approximately between 45 minutes and 1 hour 15 minutes The lysis is never permanent and is quickly followed by abundant secondary growth resistant to all bacteriophages of the Type A It attacks only the smooth elements of the culture, not touching the rough The members of this group vary widely in activity, range, virulence

and stability The virulence can be exalted, particularly if bacteriophage is freshly isolated But the great majority of the freshly isolated ChϕA are very unstable They die out within a very short period—sometimes within a few days—unless they are adapted to laboratory conditions by frequent transfers The range of virulence of some of the races of this type is often restricted to a small number of strains of vibrios, but they are comparatively easily adapted to act on the other strains of smooth cholera vibrios They do not attack the non-agglutinable vibrios, even if the latter are smooth The number of corpuscles of ChϕA usually reaches $n\times10^9$, occasionally $n\times10^{10}$ per c c The rate of multiplication of the best races is $n\times10^5$ even $n\times10^6$ in 2 hours (*see* Chapter on 'Virulence')

ChϕB The generation period of this bacteriophage on a smooth-rough culture is usually between 1 hour 15 minutes and 1 hour 45 minutes, and the lytic action of even the most active of this type is considerably slower than that of the ChϕA The lysis is seldom produced in less than three hours The lysis is also not permanent and is followed by the secondary growth, which appears later than with the Type A On the other hand, ChϕB is considerably more stable than ChϕA The range of virulence is very wide Only quite recently, after four years' of work on cholera bacteriophage, we received 12 cultures isolated in the southern part of Madras Presidency which were not attacked by our ChϕB However, after only two transfers on one of these strains, the Chϕ 146B had become adapted to it and lysed perfectly every strain of this lot ChϕB acts on both smooth and rough elements attacking also some of the non-agglutinable vibrios The number of corpuscles of ChϕB usually reaches $n\times10^9$ occasionally going up to $n\times10^{11}$ per c c The rate of multiplication is usually $n\times10^2$ in two hours

ChϕC is a slowly growing bacteriophage, with generation period of 2 hours to 2 hours 30 minutes It produces appreciable lysis only with rough cultures and even then seldom complete and followed by the usual secondary growth Very often its presence can be ascertained only by production of clearings on agar growth of vibrios This bacteriophage seems to kill vibrios without completely lysing them The platings made from a culture after some 6 to 8 hours of action on it of ChϕC, and while apparently unchanged in the test-tube, give only a few colonies on agar However, this may indicate only that ChϕC acts on the culture better on the agar surface than in the broth The range of activity of this type is very wide we have not yet met a strain of cholera vibrio which is not acted upon by our ChϕC It attacks also many non-agglutinable vibrios The number of corpuscles of this type in pure culture reaches $n\times10^9$, often $n\times10^{10}$, but in a mixture with other types its number is usually only $n\times10^8$ Its rate of multiplication is usually $n\times10^1$ in two hours

ChϕD (Pasricha) The generation period of this bacteriophage (we have only one race of this type at our disposal) is 1 hour 20 minutes to 1 hour 30 minutes on a smooth and 1 hour 30 minutes to 1 hour 40 minutes on a rough strain The lytic

action is slower than that of ChϕB but quicker than ChϕC it gives an incomplete lysis in about five hours After its action on rough culture the secondary growth develops more slowly and to a lesser extent than after the action of ChϕB The range of activity and stability of this bacteriophage have not yet been sufficiently studied Its rate of multiplication is $n \times 10^2$ in 2 hours

ChϕE (Pasricha) A very slow acting bacteriophage with generation period on rough culture of 1 hour 40 minutes to 1 hour 50 minutes seems to act only on rough elements The lytic action is very slow, but is more pronounced than with ChϕC the lysis of the rough culture is more complete than with ChϕC and the secondary growth appears with difficulty Rate of multiplication is $n \times 10^1$ in 2 hours

Combined action of choleraphages

As I have already mentioned, the lysis produced by pure-line choleraphage, however virulent it may be and whatever the number of corpuscles and vibrios used, is invariably followed by secondary growth In fact, it can be said, that the quicker the lysis, the sooner it is followed by the appearance of the secondary growth* This is so constant that if a pure-line bacteriophage culture shows the protracted appearance of secondary growth we always suspect it to be contaminated with another type, and on type-testing it, usually find our suspicions verified

Taking into consideration the fact that a secondary growth which is resistant to one type of bacteriophage remains sensitive to all the others, we might expect that the combined action of two, three, or still more of all the types will prevent the formation of secondary growth or at least retard its appearance Experience shows this to be true a combination of even two types of bacteriophage retards the appearance of secondary growth for many hours, while the combination of the best representatives of all the types may make the lysis permanent, no secondary growth appearing even after many days The action of the single and mixed types is clearly demonstrated on the Charts 1 to 5 It follows from this observation that in the practical application of bacteriophage for treatment or prevention of a disease we must use a mixture of all available types acting on the causative agent Such a mixture must have a quick acting bacteriophage (like ChϕA) in preponderance, the other types serving only the purpose of preventing the secondary growth from appearing Only then can we expect positive results I venture to express my opinion that the treatment with bacteriophage of diseases such as, for example,

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* This may be attributed simply to the exhaustion of the medium The later the lysis the longer the normal bacteria develop exhausting the medium and saturating it with products of metabolism This may hinder the development of the secondary growth

CHART 1

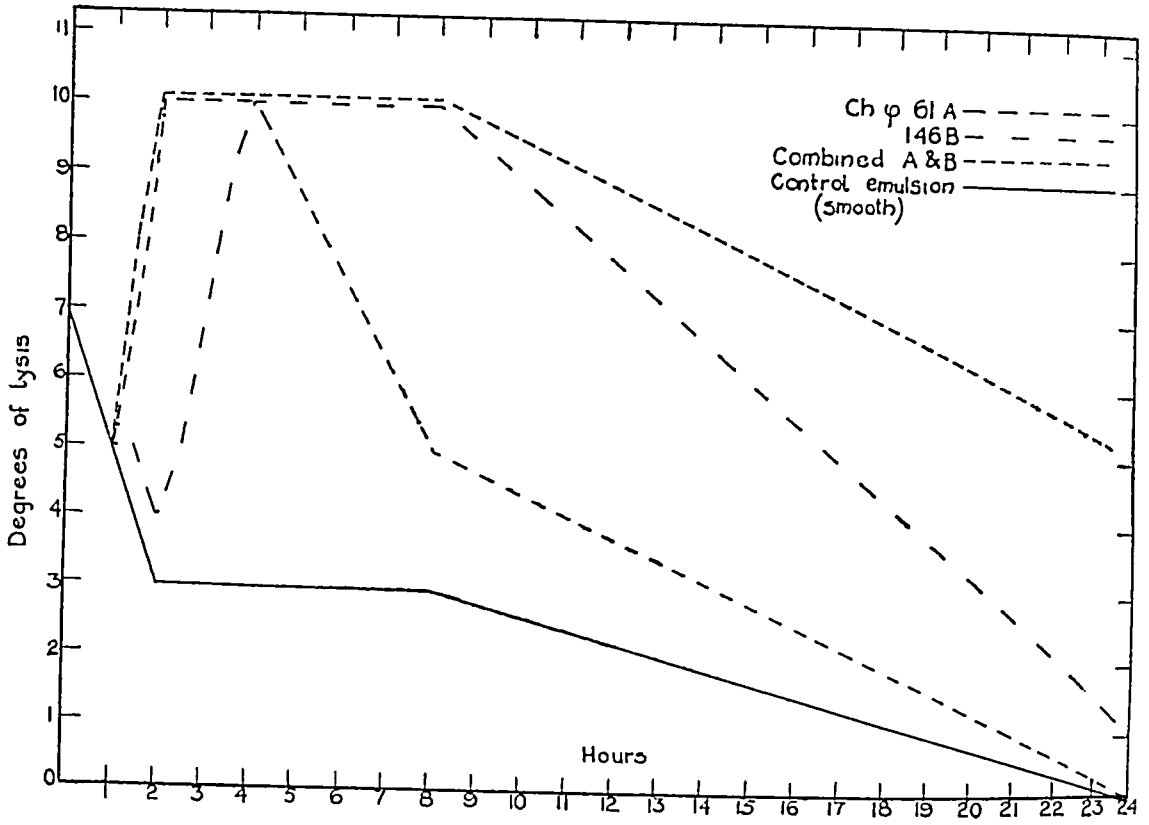


CHART 2.

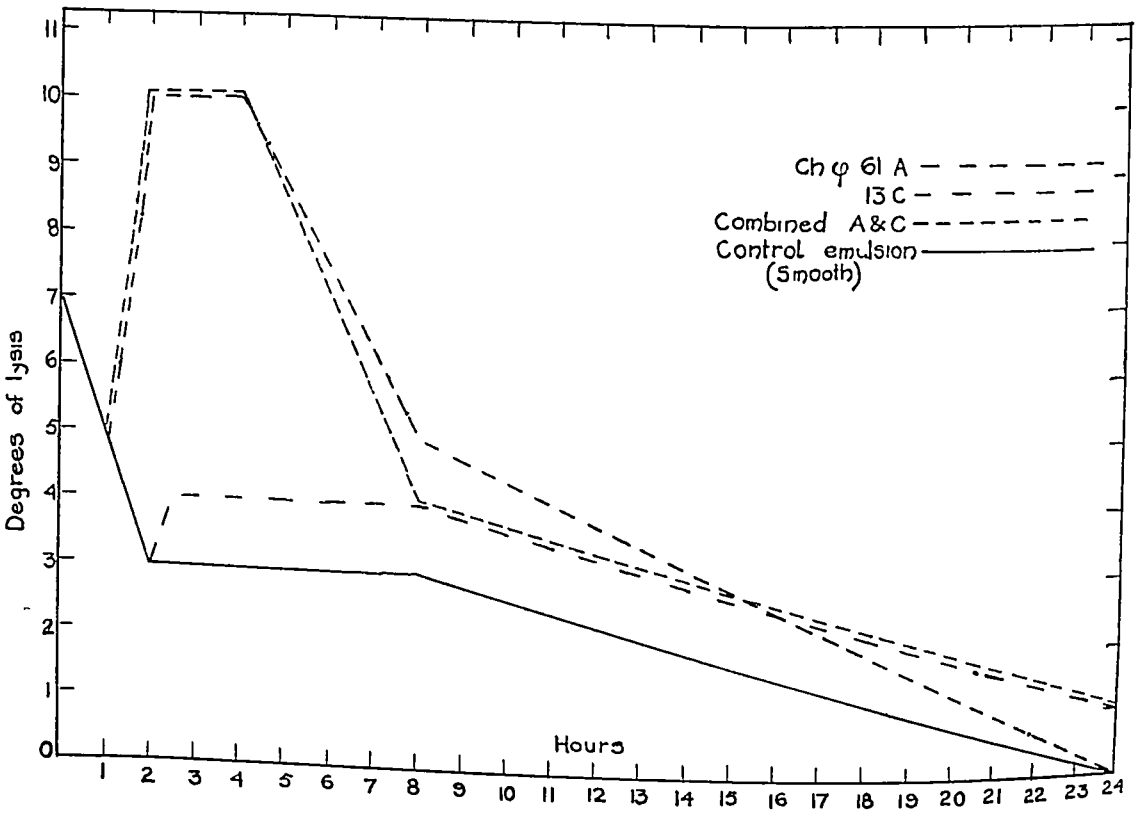


CHART 3

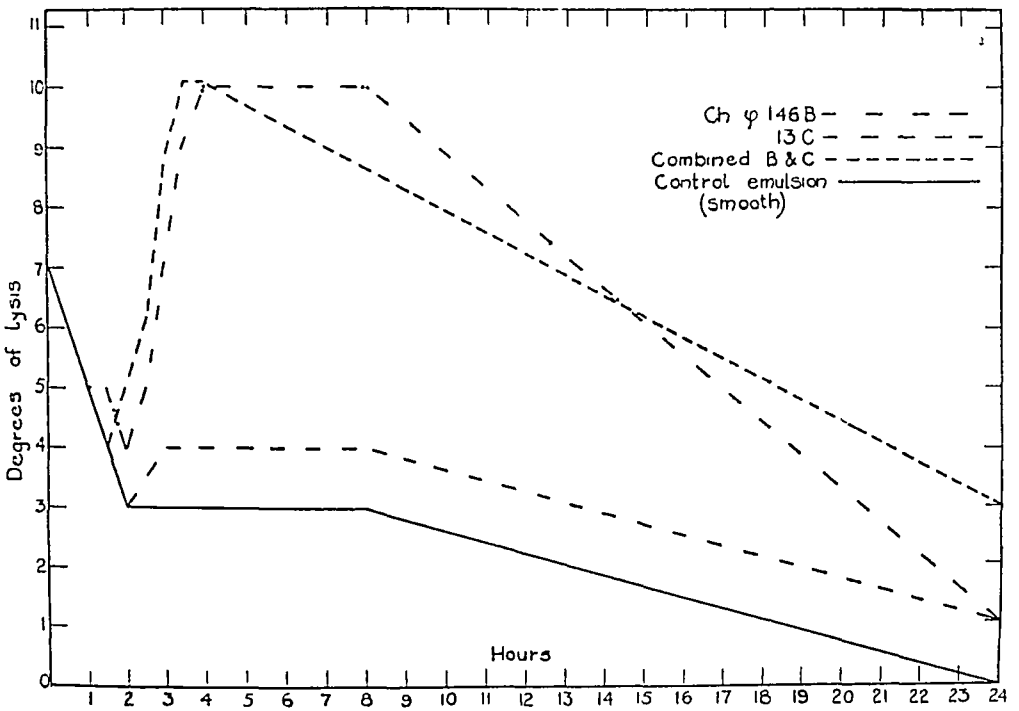


CHART 4

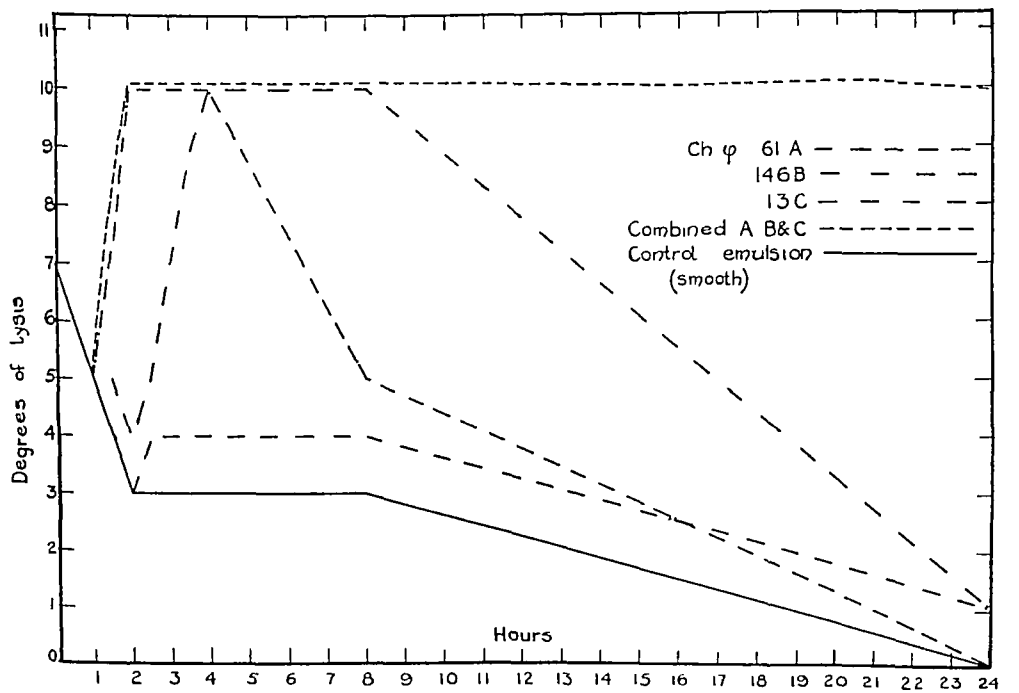
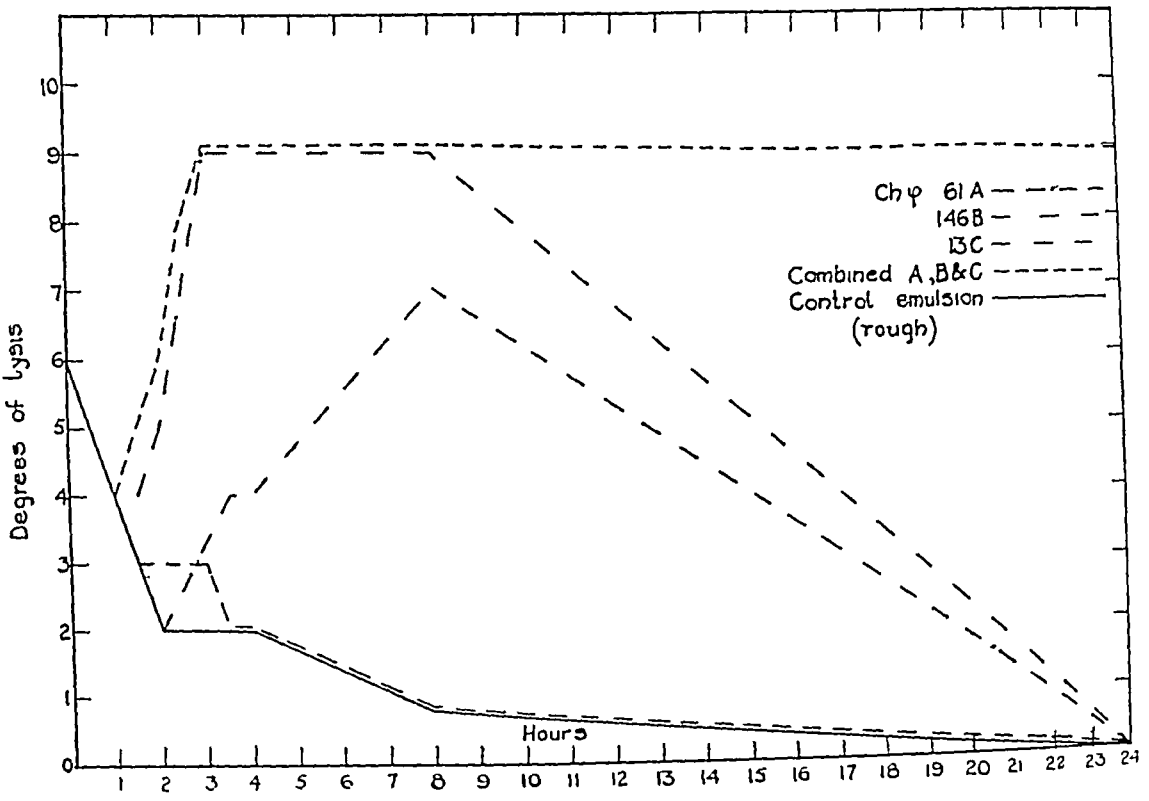


CHART 5



typhoid fever in men or paratyphoid infections in mice (Topley) lacked in success probably because the different types of corresponding bacteriophages were not recognized and the mixture of bacteriophages used was incomplete

After this description of the type characteristics of bacteriophage, I must emphasize again the absolute necessity of studying bacteriophages only in pure-line cultures, of studying first of all the 'type characters' and only then the individual character of every race This introduces a system in our studies and only a system can put in order the mass of accumulated observations Otherwise we are bound to get contradictory and misleading results

TYPE-TEST

The reciprocal compensatory action of the different types of bacteriophage on the secondary growth of bacteria gave us the possibility of developing a new method in the study of bacteriophage This method makes it possible —

1 To determine the type of a bacteriophage culture

2 To ascertain its purity

3 If different types are present, to determine them

1 To find out whether a bacterial culture is contaminated or is in symbiosis with bacteriophage, and to determine the type of this bacteriophage—(ultra-purity test)

For determination of the type of bacteriophage I developed the following method called 'type-test' In brief the technique of this test is to spread separately the secondary growths resistant to the standard types of bacteriophage and to the bacteriophage under examination, and to superimpose on each spreading also separately, in drops, filtrate containing the bacteriophage under examination and filtrates containing pure-line cultures of the standard types

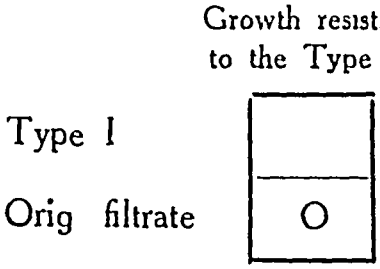
To perform this test it is necessary to have pure-line cultures representing all the types of bacteriophage known to act on the particular bacterium If such standard cultures of bacteriophage are not available the following technique is recommended to obtain them —

Isolation of standard types —We start with a filtrate containing the bacteriophage or bacteriophages under investigation By repeated spreadings and isolation from a single clearing well separated from the others, we obtain our first pure-line standard culture of bacteriophage To be sure of its purity it is better to make at least five successive isolations from the single clearing, as indicated in Part I under 'Isolation of a pure-line types' After that the chances that it is still not pure are the same as in bacteriological work where we obtain by the same method of repeated isolation a standard pure culture of bacteria The next step is to find out whether our original filtrate contained, besides that isolated—let us call it Type I—another type of bacteriophage active against the same bacteria For this we inoculate a bacterial emulsion with the Type I already obtained When the secondary growth appears we spread it on the surface of agar in a Petri dish* Then we filter the culture of the Type I and superimpose a drop of it and the drop of the original filtrate on the surface where the secondary growth is spread If our original filtrate contained only

* The majority of experimenters used for cross testing with other bacteriophages, the resistant culture isolated from a single colony on a plating of secondary growth, and usually selected one which was free from bacteriophage In my opinion this procedure for our purpose (type test and its different uses) must be avoided Experience shows that the secondary growth which has become resistant, say, to the Type I bacteriophage, contains some cells which possess a natural resistance also to the action of the other types This is proved by the presence of resistant colonies of bacteria in the clearings produced, say, by Type II on the spreading of growth resistant to Type I Sometimes the number of such colonies is considerable For example, isolating a colony resistant to Type I we may pick up one which is at the same time resistant to the Type II Moreover, some resistant cultures, freed from bacteriophage, might regain their sensitivity to this bacteriophage quite soon This would lead us to erroneous conclusions when using such a culture for type testing Some of Bail's (*loc cit*, pp 124 125) experiments suggest it On the other hand, if the secondary culture is used *in toto*, the action of another type is in the majority of cases quite clear

one type of bacteriophage which we isolated in pure-line culture, we will have on the agar an even unbroken layer of bacterial growth the secondary growth resistant to the isolated bacteriophage will be resistant to both filtrates In that case I would advise using the whole original filtrate as the standard bacteriophage culture, and not the repeatedly re-isolated one by repeated isolation we can lose some of the valuable and interesting properties of that particular race of bacteriophage

If on the contrary our original filtrate contained two *or more* different types of bacteriophage, the picture on agar will be —



because the secondary growth is resistant only to the isolated Type I and a bacteriophage of another type present will act on it In that case we proceed with isolation of the second type from the same original filtrate For that purpose we plate an adequate dilution of this filtrate along with the culture on three of the four quadrants of a Petri dish so as to obtain discrete clearings On the fourth quadrant we plate the pure-line culture of the isolated Type I for comparison A search is made on the first three quadrants for clearings which show some difference in morphology (and not only in size) from those of the Type I If no clearings of different morphology are present, it may mean either that the agar is not adequate to show the morphological difference or that the other type of bacteriophage is present in too small numbers to show itself in this dilution The methods of isolation in the latter case will be given later in this section (*see* page 1154) and in a subsequent paper If a different clearing is found it is picked up into bacterial emulsion From this tube containing the newly isolated bacteriophage platings and repeated isolations from single clearings are made and the secondary growth after the last isolation is used for spreading Besides this secondary growth resistant to the newly isolated Type II, secondary growths resistant to Type I and to both Type I and Type II combined are prepared and spread on the agar, forming in all three vertical columns The three tubes from which the spreadings were made are filtered* and the filtrates superimposed in drops on the spreadings If our original filtrate contained only two types of

* It often saves time if two tubes are prepared for each One is filtered, the other is kept to be used in case the first spreadings after 24 hours do not yet give sufficient growth on agar

bacteriophage and we have succeeded in the isolation of the second one, the picture on agar will be —

		Growth resistant to types		
		I	II	I + II
Filtrates	I		O	
	II	O		
	I + II	O	O	
Orig filtrate		O	O	

We see here the reciprocal action of our two types on their secondary growths and that the original filtrate and the secondary growth resistant to it behave like a mixture of Type I and Type II only Type II present in the original filtrate acts on the secondary growth of Type I, and Type I also present there acts on the secondary growth of Type II If, on the contrary, our original filtrate contained more than two types we will have —

		Growth resistant to types		
		I	II	I + II
Filtrates	I		O	
	II	O		
	I + II	O	O	
Orig filtrate		O	O	O

The clearing on the secondary growth resistant to both Types I and II denotes the presence of a third type in the original filtrate In such a case we proceed again with plating of the filtrate along with the two isolated bacteriophages, trying to find the third type— and so on

When examining a new filtrate where we suspect the presence of a new type of bacteriophage we proceed in exactly the same manner as described, spreading the secondary growths resistant to the pure types already obtained along with that resistant to all of them together It is of advantage in that case to spread

also the growth resistant to the whole new filtrate as that can give direct indications of the types present and already known to us For instance, if we are already in possession of three types of bacteriophage and the filtrate under investigation contains no new types but only Type II, we will have —

Filtrates	Growth resistant to I	Growth resistant to II	Growth resistant to III	Growth resistant to I+II+III	Growth resistant to New filt
I		O	O		O
II	O		O		
III	O	O			O
I + II + III	O	O	O		O
New filt.	O		O		

Whereas if a new Type IV is also present, the new filtrate will produce a clearing on II and on the I + II + III secondary growth

The type-test is performed in a similar way As an example I will take again the case of choleraphage At present we know already five types of choleraphage But to simplify the explanations of the technique I will take into consideration only three types—ChϕA, ChϕB and ChϕC, only occasionally referring to the other two types also More types mean only more test-tubes and more spreadings—the principle remains the same In the tables I have given the results of using all five types

The type-test proper —To determine the type of bacteriophage present, or to ascertain the purity of a bacteriophage culture, or in the case of a mixture, to determine the types of bacteriophage present —

(1) Take four tubes containing emulsion of cholera vibrio in broth, inoculate three of them with our three standard pure-type bacteriophages separately and the fourth with the filtrate under investigation

(2) When the secondary growth appears in these tubes, spread it from each tube separately on the surface of agar in a Petri dish in four vertical columns

(3) Filter all four tubes (*see* footnote on page 1140) Place a drop of each filtrate on every secondary growth spread, in four horizontal rows.

TABLE I

	A	B	C	D	E	?
A		O	O	O	O	
B	O		O	O	O	O
C	O	O		O	O	O
D	O	O	O		O	O
E	O	O	O	O		O
?		O	O	O	O	

A

	A	B	C	D	E	?
A		O	O	O	O	O
B	O		O	O	O	
C	O	O		O	O	O
D	O	O	O		O	O
E	O	O	O	O		O
?	O		O	O	O	

B

	A	B	C	D	E	?
A		O	O	O	O	O
B	O		O	O	O	O
C	O	O		O	O	
D	O	O	O		O	O
E	O	O	O	O		O
?	O	O		O	O	

C

	A	B	C	D	E	?
A		O	O	O	O	O
B	O		O	O	O	O
C	O	O		O	O	O
D	O	O	O		O	
E	O	O	O	O		O
?	O	O	O		O	

D

	A	B	C	D	E	?
A		O	O	O	O	O
B	O		O	O	O	O
C	O	O		O	O	O
D	O	O	O		O	O
E	O	O	O	O		
?	O	O	O	O		

E

	A	B	C	D	E	?
A		O	O	O	O	
B	O		O	O	O	
C	O	O		O	O	O
D	O	O	O		O	O
E	O	O	O	O		O
?	O	O	O	O	O	

A+B

TABLE I—*contd*

	A	B	C	D	E	?
A		O	O	O	O	
B	O		O	O	O	O
C	O	O		O	O	
D	O	O	O		O	O
E	O	O	O	O		O
?	O	O	O	O	O	

A + C

	A	B	C	D	E	?
A		O	O	O	O	
B	O		O	O	O	O
C	O	O		O	O	O
D	O	O	O		O	
E	O	O	O	O		O
?	O	O	O	O	O	

A + D

	A	B	C	D	E	?
A		O	O	O	O	
B	O		O	O	O	O
C	O	O		O	O	O
D	O	O	O		O	O
E	O	O	O	O		
?	O	O	O	O	O	

A + E

	A	B	C	D	E	?
A		O	O	O	O	O
B	O		O	O	O	
C	O	O		O	O	
D	O	O	O		O	O
E	O	O	O	O		O
?	O	O	O	O	O	

B + C

	A	B	C	D	E	?
A		O	O	O	O	O
B	O		O	O	O	O
C	O	O		O	O	
D	O	O	O		O	
E	O	O	O	O		O
?	O	O	O	O	O	

C + D

	A	B	C	D	E	?
A		O	O	O	O	O
B	O		O	O	O	O
C	O	O		O	O	O
D	O	O	O		O	
E	O	O	O	O		
?	O	O	O	O	O	

D + E

	A	B	C	D	E	?
A		O	O	O	O	
B	O		O	O	O	
C	O	O		O	O	
D	O	O	O		O	O
E	O	O	O	O		O
?	O	O	O	O	O	

A+B+C

	A	B	C	D	E	?
A		O	O	O	O	
B	O		O	O	O	O
C	O	O		O	O	O
D	O	O	O		O	
E	O	O	O	O		
?	O	O	O	O	O	

A+D+E

	A	B	C	D	E	?
A		O	O	O	O	O
B	O		O	O	O	
C	O	O		O	O	
D	O	O	O		O	
E	O	O	O	O		O
?	O	O	O	O	O	

B+C+D

	A	B	C	D	E	?
A		O	O	O	O	O
B	O		O	O	O	O
C	O	O		O	O	
D	O	O	O		O	
E	O	O	O	O		
?	O	O	O	O	O	

C+D+E

	A	B	C	D	E	?
A		O	O	O	O	
B	O		O	O	O	
C	O	O		O	O	
D	O	O	O		O	
E	O	O	O	O		O
?	O	O	O	O	O	

A+B+C+D

	A	B	C	D	E	?
A		O	O	O	O	
B	O		O	O	O	
C	O	O		O	O	
D	O	O	O		O	
E	O	O	O	O		
?	O	O	O	O	O	

A+B+C+D+E

Reading the results—Next day, after incubation, when the growth of resistant vibrios has appeared on the agar, we will see (*a*) on the secondary growth resistant to ChϕA clearings on the spaces where the drops of filtrates containing ChϕB and ChϕC were placed, (*b*) on the secondary growth resistant to Chϕ*B* clearings produced by ChϕA and ChϕC, and (*c*) on the secondary growth of ChϕC clearings produced by ChϕA and ChϕB

If we now study the fourth column of the secondary growth resistant to the bacteriophage under investigation, we find that —

1 If the bacteriophage under investigation was a pure culture of one type, only two of the standard types of bacteriophage will produce clearings on it thus

(*a*) If it was ChϕA, ChϕB and ChϕC will give clearings and not ChϕA

(*b*) If it was ChϕB, clearings will be produced by ChϕA and ChϕC and not by ChϕB,—and so on

The diagnosis is confirmed by observation of the action of the filtrate of the bacteriophage under investigation on the secondary growth of the standard types (the bottom row) If the filtrate under investigation contains only one type of bacteriophage it will act on the secondary growths of the two standard types only Thus, if it was ChϕA, it will produce clearings on the secondary growths of the standard ChϕB and ChϕC and not on the standard ChϕA—its own type

2 If the filtrate under investigation is a mixture of two or more bacteriophages, it is obvious that it will produce clearings on all the standard secondary growths The types which this mixture contains will be recognized by the action of the standard bacteriophages on the secondary growth of the mixture under investigation only bacteriophages of types *not* present in the mixture would be able to produce the clearings If the mixture contains all the types, none of the standard bacteriophages will act on it

Some of the combinations are shown in Plates LIII and LIV using three types of bacteriophage and Plate LII, fig 13, and Table I using all five types

Short-cut method of the type-test—For a short-cut method the principle of determination of the presence of bacteriophage and of the type to which it belongs by examination of the resistance of the secondary growth to the standard types, can be used when it is necessary to examine a large number of filtrates In that case a large amount of filtrate—say 5 c c —is inoculated into 20 c c of broth with a small amount of a ultra-pure sensitive culture—0 5 to 1 c c of 16 hours old growth in broth or peptone water After incubation, when there is sufficient secondary growth, it is spread on the surface of agar and on it the all available standard types of bacteriophage are deposited in drops The standard types which *do not* act on this secondary culture indicate the types of bacteriophage present in the filtrate However, I strongly advise not relying upon this short-cut method absolutely but, before final selection of the races of bacteriophage for work, to control them by means of the standard type-test

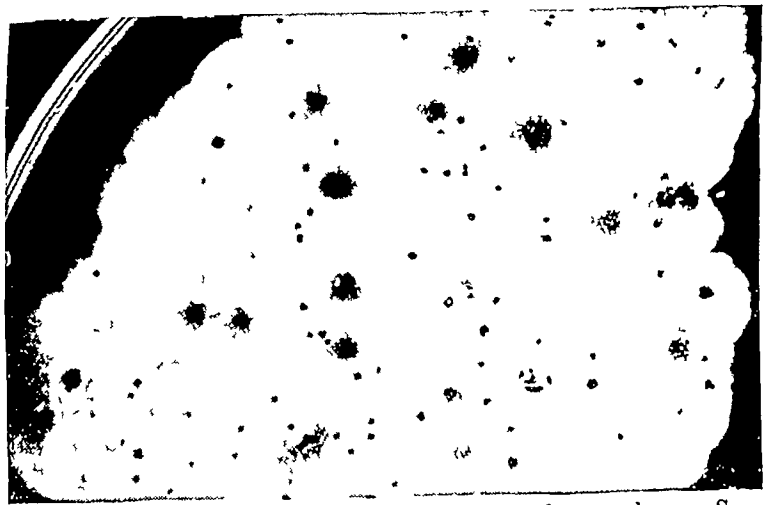


Fig 10 'Dissociation' phenomenon amongst bacteriophages Same bacteriophage as on Fig 9 Both types of clearings are produced by ChϕA Magnification × 2

Fig 11 'Dissociation' phenomenon amongst bacteriophages ChϕB showing different morphology of clearings with and without white ring Magnification × 2

Fig 9 'Dissociation' phenomenon amongst bacteriophages The top group of clearings is produced by a drop of a filtrate containing ChϕA soon after isolation from stool The bottom group—same bacteriophage after few transfers in laboratory Both kinds of clearings—the small clear-cut and one with a large zone—

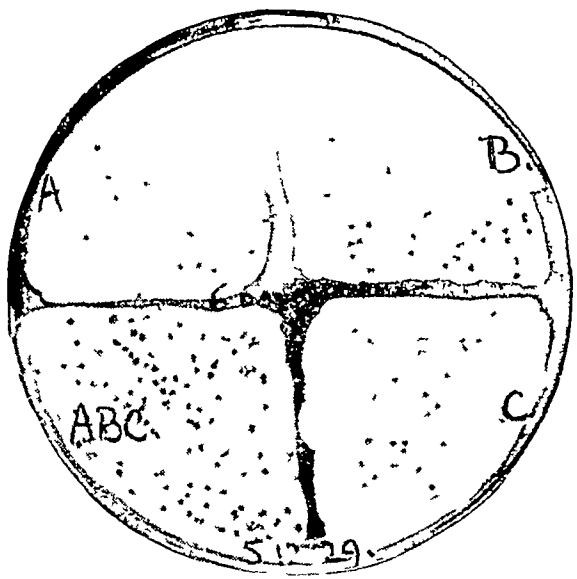


Fig 12 Three types of choleraphage—ChϕA, ChϕB and ChϕC—separate and mixed together

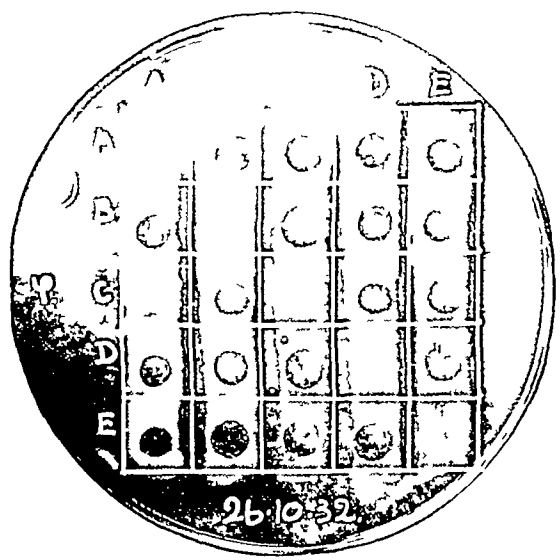


Fig 13 Complete type-test with five types of

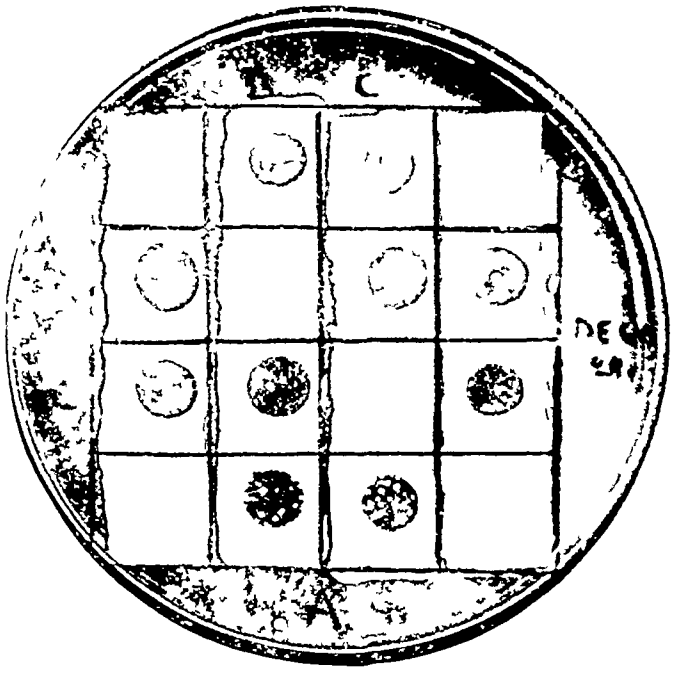


Fig. 14.

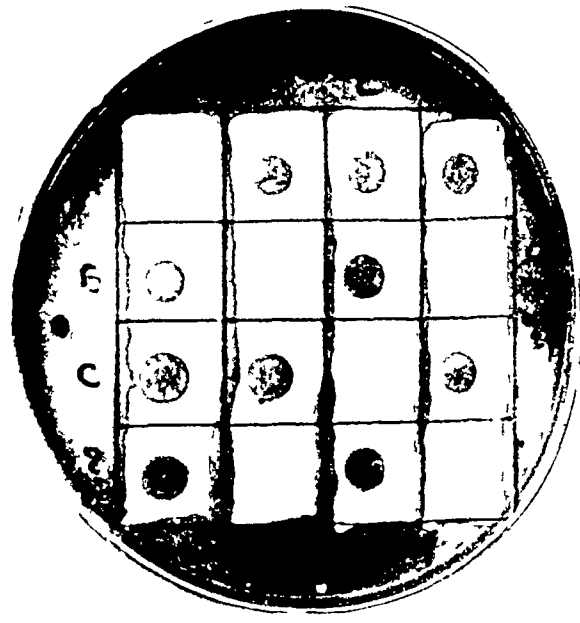


Fig. 15.

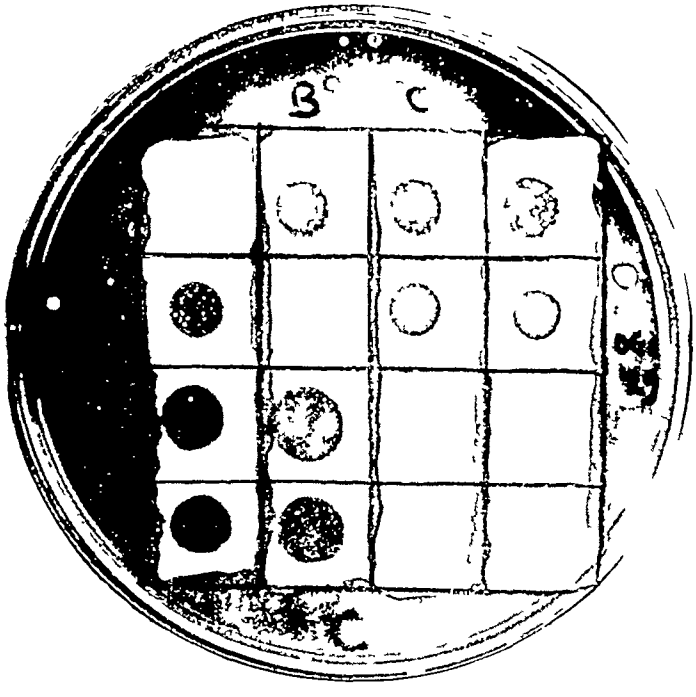


Fig. 16

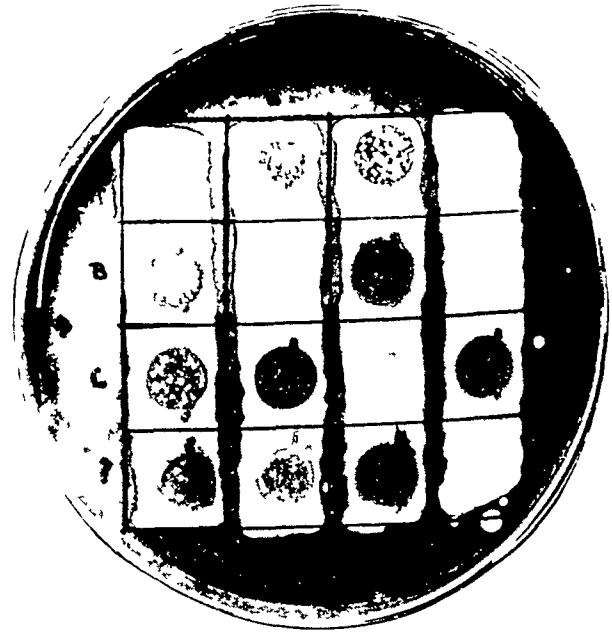
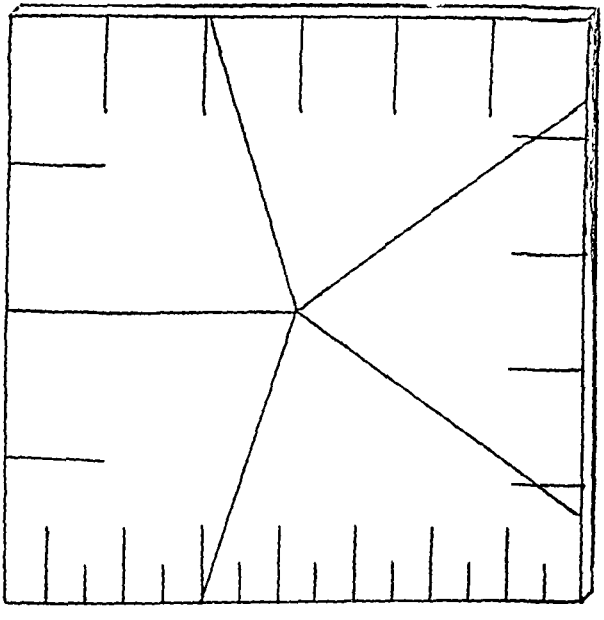


Fig 17

Figs 14 to 17. Type-test with three types of choleraphage

The technique with bacteriophages other than choleraphage will be the same The secondary growths resistant to the available types are to be spread along with the growth resistant to the bacteriophage or bacteriophages under investigation, and their filtrates superimposed on all of them If we are not sure that we possess all the types of bacteriophage active against the bacterium we are working with, it is necessary to spread also along with the others the growth resistant to all the available types together only then can a new as yet undiscovered type reveal its presence by acting on this growth The easier way to prepare such a combined resistant growth is



TEXT FIGURE —Glass stencil

not to inoculate a normal emulsion of bacteria with the mixture of bacteriophages, but to obtain first the resistant growth to the single types and then to mix one c c of each in 20 c c of broth A partial lysis will follow, then turbidity will start to increase If a spreading on agar does not show sufficient growth, this can be improved by one or two transfers of 1 c c in 20 c c of broth With some bacteriophages, e g , dysenteryphages, not only is it difficult to obtain a growth resistant to all the types available but it is even difficult to obtain one resistant to some of the single types I would in this case recommend the use of a very large amount of broth—1 to 2 litres The proportion of bacteria and bacteriophage can be arranged so that the lysis appear considerably later

and bacteria continue to grow longer As in this case we bring into play a larger number of bacteria, we have more chance of finding amongst them a bacterium which will offer resistance to the bacteriophage and grow, forming the secondary resistant growth The alternative will be to use instead of this type-test the serum type-test which will be described in a subsequent paper

I recommend making the spreadings in the following way With a glass pencil a square is drawn on the back of the Petri dish This square is divided into the necessary number of vertical columns and horizontal rows To make it neatly, I recommend the use of a square glass stencil, on the sides of which are marked different divisions—thirds and sixths, fourths and fifths (*see* Text-figure) For a 12 cm Petri dish a square of 7 5 cm is convenient The spreading of the culture is made with a 'spreader'—a large flat perpendicular loop, bent conveniently so as to make the spreadings under the partly lifted cover of the dish [Plate XLVIII, fig 11 (facing page 1118) and Plate LIV, fig 21] The spreader is made by bending the nichrom wire round a metal plate 10 mm to 12 mm large and 1 mm thick[*]

Ultra-purity test for bacterial cultures

Many authors have described the so-called 'lysogenic cultures', i e, cultures which reveal the lytic action if brought into contact with another strain of bacteria sensitive to the action of bacteriophage Theories have been propounded, explaining the origin of bacteriophage in this case as 'microbic antagonism', ascribing the appearance of bacteriophage not to its presence in the 'lysogenic culture' but to the peculiar action of that culture on another Such theories were forced into existence by the difficulty of proving the presence of bacteriophage in the 'lysogenic culture' without bringing it into direct contact with a culture sensitive to the action of bacteriophage We believe that bacteriophage can be so closely associated with bacteria (sometimes probably, as d'Herelle insists, even living in symbiosis with these bacteria) that it cannot be revealed by simple filtration of broth culture But if this culture is brought into contact with bacteria sensitive to bacteriophage action, bacteriophage contaminating the 'lysogenic culture' will start to propagate on these bacteria, imitating the 'microbic antagonism' Many complicated and drastic methods—not always successful—have been devised to reveal bacteriophage in such 'lysogenic cultures' without introducing a sensitive culture

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[*] A convenient set of calibrated wires and plates to make loops and spreaders was made after my design by Les Etablissements Leune, 28 bis rue Cardinal Lemoine, Paris

Accepting the living nature of bacteriophage, we also accept d'Herelle's interpretation of the nature of the 'lysogenic cultures' they are cultures contaminated with bacteriophage, or symbiotic cultures of bacteria bacteriophage In the last case bacteriophage may be particularly closely associated with the bacterial cell and it would be difficult to separate it by ordinary means Such cultures will be actually nothing else but the secondary growth, resistant to the action of bacteriophage contaminating it, as well as to all bacteriophages belonging to the same type as the contaminating one If, as sometimes happens this culture is still sensitive to bacteriophage of the same type it is due to extremely low virulence of the contaminating bacteriophage on the one hand and on the other to the high virulence of the bacteriophage so found sensitive But such a culture will be always sensitive to bacteriophages of other types Then, by means of such bacteriophage we can easily disintegrate bacterial cells, lyse them, and liberate the contaminating bacteriophage

With this idea in mind I have devised the following technique for discovering the bacteriophage contaminating or living in symbiosis with a bacterial culture —

Experience shows that very seldom, if ever, is a culture contaminated with two different types of bacteriophage Therefore it is enough to try and lyse the suspected culture with any two types of bacteriophage active against such a culture But to be on the safe side I would advise using at least three different types This will provide additional evidence as to the type of contaminating bacteriophage The races of bacteriophage used should preferably be of good activity acting rapidly

Technique —To four tubes with 20 c c of broth are added, say, one c c each of the culture under investigation Three of them are inoculated separately with the three chosen types of bacteriophage The fourth tube is left to grow without the addition of bacteriophage The tubes are examined every half hour and each of them is filtered as soon as the lysis reaches its maximum The tube without additional bacteriophage is filtered at the same time as the tube last to be lysed Each filtrate is transferred *in toto* into a flask with 50 c c of broth and to which is added 2 c c of a sensitive culture of corresponding bacteria known to be ultrapure The flasks are left until secondary growth appears when a test based on the same principle as the type-test is made with all of them

As an example I will take again the case of a cholera culture, contaminated, as happens in great majority of cases, with ChϕA, and in which the contaminating bacteriophage cannot be discovered by ordinary means All the secondary growths, including that of the flask without additional bacteriophage, are spread on the surface of the agar in vertical columns along with a normal sensitive culture On each column a drop of the same three standard types of bacteriophage, as well as

the filtrate of the culture grown without added bacteriophage, is superimposed The result after incubation will be —

		Secondary growth of (A)+A	Secondary growth of (A)+B	Secondary growth of (A)+C	Investigated culture	Normal culture
Standard bacteriophages.	A					O
	B	O		O	O	O
	C	O	O		O	O
Filtrate of investigated culture.						†

* A clearing will appear here if the contaminating bacteriophage is of a very low virulence

† A clearing will appear here if the contaminating bacteriophage was not too closely associated with the culture and could be found free in the ambiant liquid without bacterial cells being lysed by another bacteriophage

The absence of the action of the standard ChϕA on all three secondary growths indicates the presence of the ChϕA, contaminating the culture under examination

The test in the arrangement described is designed to meet possible objections by the advocates of the theory of 'microbic antagonism' and 'lysogenic cultures' As we are convinced that the 'antagonism' is produced by the contaminating bacteriophage, our routine test for ultra-purity is more simple

Four tubes with the bacterial emulsion under investigation—(one c c of 16 hours' old culture in broth or peptone water to 20 c c of broth)—are prepared, and three of them are inoculated with three different types of virulent bacteriophages, to the fourth being added 0 5 c c of known ultra-pure and sensitive culture After three hours to the three tubes inoculated with bacteriophages is also added 0 5 c c of the ultra-pure sensitive strain to provide the 'food' for the bacteriophage possibly liberated After 24 or 48 hours, when the secondary growth appears in the first three tubes it is spread on the agar as in the type-test, along with the known ultra-pure strain The standard suspensions of all five types of bacteriophage and the filtrate from the fourth tube (culture under investigation plus ultra-pure strain) are deposited on each spreading To make sure that we do not miss the liberated bacteriophage, if it is present in small numbers, the

tubes can be filtered and one transfer made on the sensitive ultra-pure strain, using one c c of the filtrate Then the secondary growths from these tubes are used for the test The result, if we take the same example, will be —

	Secondary growth of (A) + A	Secondary growth of (A) + B	Secondary growth of (A) + C	Investigated culture	Normal culture
A					O
B	O		O	O	O
C	O	O		O	O
D	O	O	O	O	O
E	O	O	O	O	O
Culture filtrate					*

* Here the clearing will appear only if the contaminating bacteriophage is easily separated from the culture under examination

The normal growth and the filtrate without added bacteriophage are used only to see to what extent the contaminating bacteriophage was associated with bacteria, and whether it would be possible or not to detect it without preliminary lysis by other bacteriophages to liberate it

One-type-sensitive cultures

The further logical development of the same principle was to obtain what we call ' One-Type-Sensitive Culture ' or ' Minus One-Type Secondary Growths ' As the name suggests, these cultures are prepared by inoculating a normal bacterial emulsion with all the available standard types of bacteriophage but one The secondary growth resulting will be sensitive only to that missing type (Plate LV, fig 22) By using this culture for plating of a mixture of different types of bacteriophage we hoped to be able to count separately the number of corpuscles of each type of bacteriophage present Experience showed that this method has no practical value In the section on plating and counting of bacteriophage corpuscles I have already mentioned the influence of the culture on the number of clearings appearing and the impossibility of determining the actual number of bacteriophage corpuscles When using for plating the ' One-Type-Sensitive ' (OTS) cultures this influence on the number of clearings appearing is still more pronounced

than in the case of ordinary cultures For instance, if we prepare ' Only C Sensitive ' culture (' oCs '—culture resistant to all types of choleraphage with exception of the Type C) and use it for the plating of a suspension of ChϕC corpuscles, using a normal smooth-rough culture of cholera vibrio as a control, we may obtain *ten times* more clearings on ' oCs ' than on the normal Besides this, the difference varies with each ' OTS ' culture prepared (Plate LV, figs 23 to 25)

However, the ' OTS ' cultures proved to be very useful in other ways in the investigation of bacteriophage

For cholera work we found the following method to be the best for obtaining ' OTS ' cultures Tubes of normal cholera emulsion are inoculated separately with each standard type of choleraphage available When the secondary growths appear mixtures of them are made so as to obtain combinations of secondary growths of all types minus one Thus, for example, for ' oAs ' we combine the secondary growth of choleraphages B, C, D and E , for ' oBs ', choleraphages A, C, D and E, and so on The mixture is made by adding 1 c c of each culture to 20 c c of broth There will be a partial lysis followed by the ' tertiary ' growth, which will be sensitive only to the missing type For keeping these cultures are better transferred on agar slopes If the growth on agar is poor, one more transfer will improve it In this case it is advisable to cover the surface of agar, before seeding it with the culture, with a corresponding mixture of bacteriophages, that is to say ' minus one type ' mixture On these slopes the ' OTS ' cultures can be kept for about a fortnight, when it becomes advisable to prepare a fresh set, as on longer standing they have a tendency to become partially resistant to the missing type also This is particularly pronounced with cultures in broth Success in the preparation of these ' OTS ' cultures depend on the quality of the strain of bacteria used some strains are particularly good for the purpose, giving clear-cut results, while some are quite unsuitable Therefore I would advise trying at least six different strains (of different periods of isolation, different degrees of S-R dissociation,—and so on), and using only one which gives clear-cut results with all types of bacteriophage

For use 20 c c of broth is comparatively heavily seeded from the agar slant The growth can be used after a short incubation of some 4 to 8 hours and is still good after 48 hours After this it is better to renew it To perform various tests possible the ' OTS ' cultures are spread by means of the ' spreader ' [Plate XLVIII, fig 11, (facing page 1118)] in vertical columns on an agar surface in a large Petri dish, and drops of the filtrates under investigation are deposited on them

In this way the ' OTS ' cultures can be used for a short-cut method of determination of the types of bacteriophages present in a filtrate Table II gives the results which are obtained with filtrates containing the five choleraphages and the twenty possible combinations of mixtures However, using this method it is easy to miss a bacteriophage if it is present in small numbers, theoretically in less than one in each drop Therefore this method is not to be relied upon absolutely E

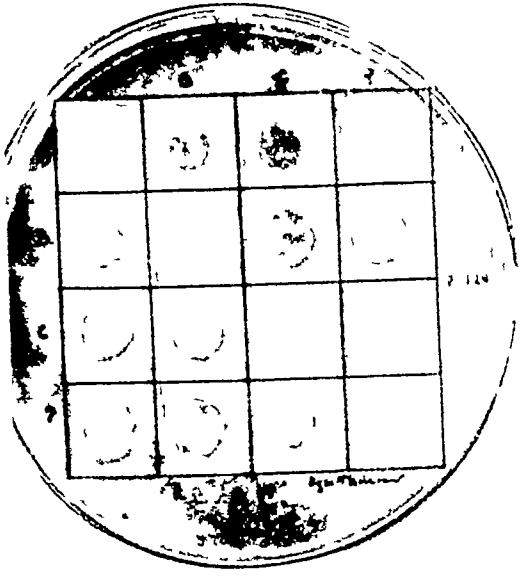

Fig 18,

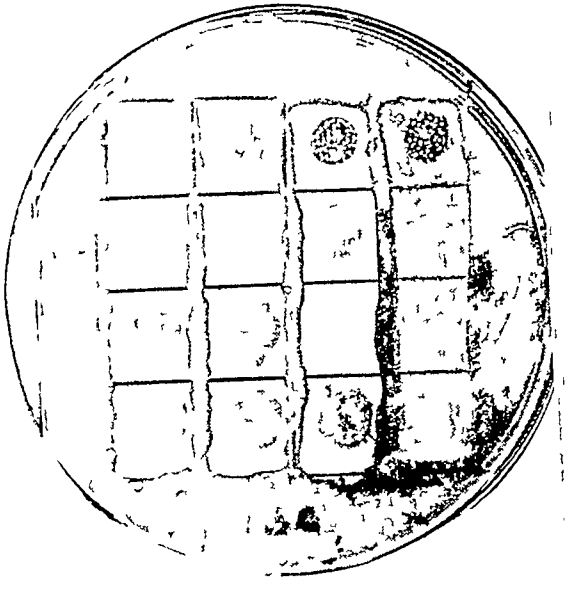

Fig. 19

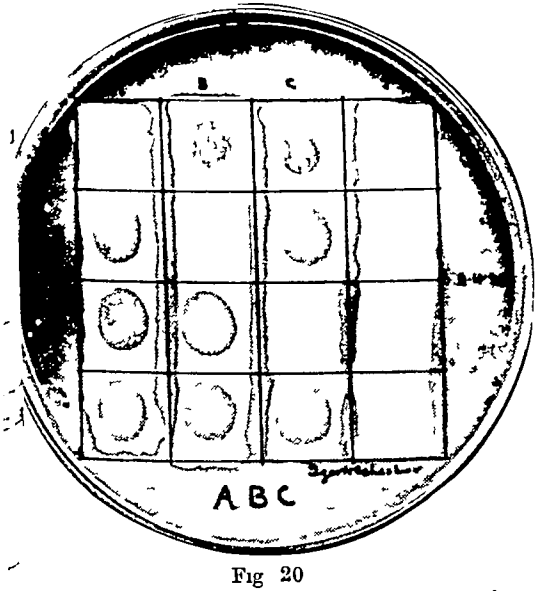

Fig 20

Figs 18 to 20 Type test with three types of choleraphage

Fig 21 Spreading the cultures for type-test

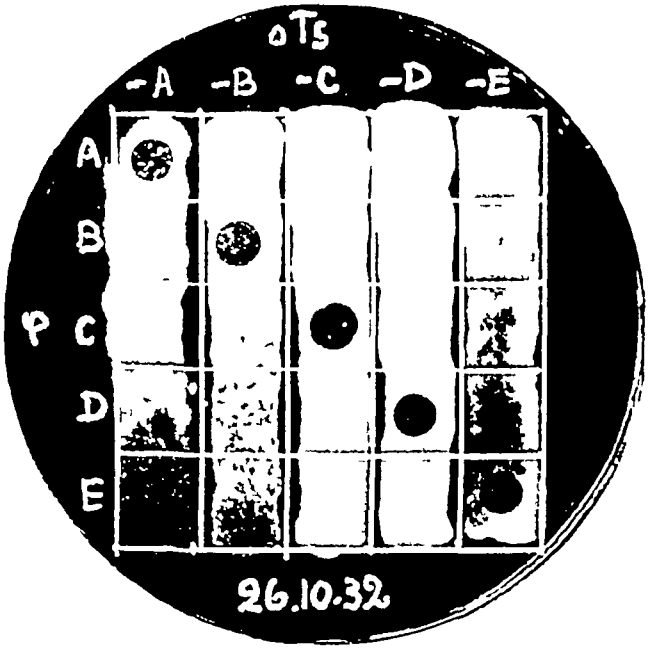

Fig 22 Complete test with 'One Type Sensitive' cultures

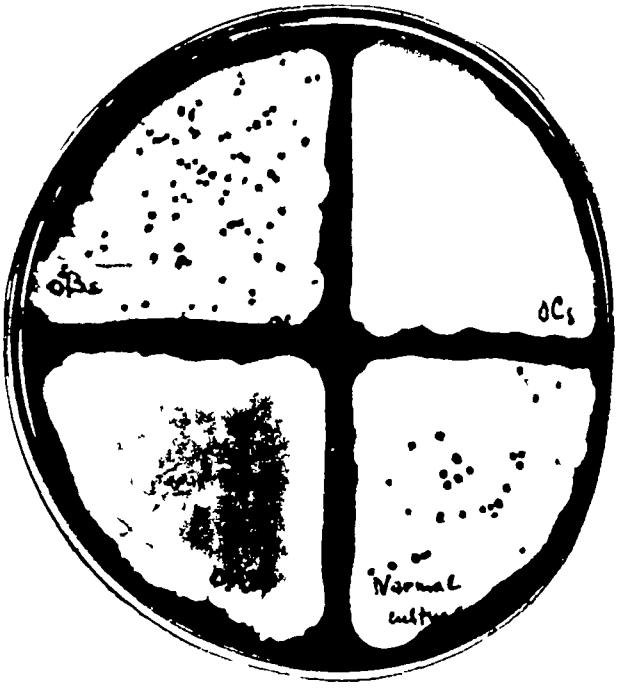

Fig 23 Control plating of pure $Ch\phi B$ with normal 'oAs', 'oBs' and 'oCs' cultures The 'oAs' and 'oCs' cultures do not allow $Ch\phi B$ to develop clearings, while 'oBs' culture in the same dilution of bacteriophage suspension shows more than twice the number of clearings as compared with normal culture

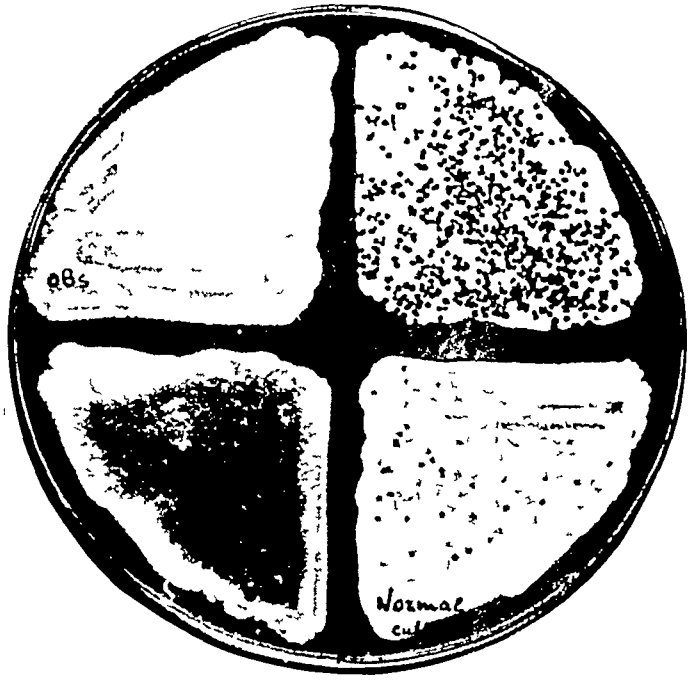

Fig 24 Control plating of pure $Ch\phi C$ with normal 'oAs', 'oBs' and 'oCs' cultures The 'oCs' culture shows many times more clearings with the same dilution of $Ch\phi C$ than the normal culture

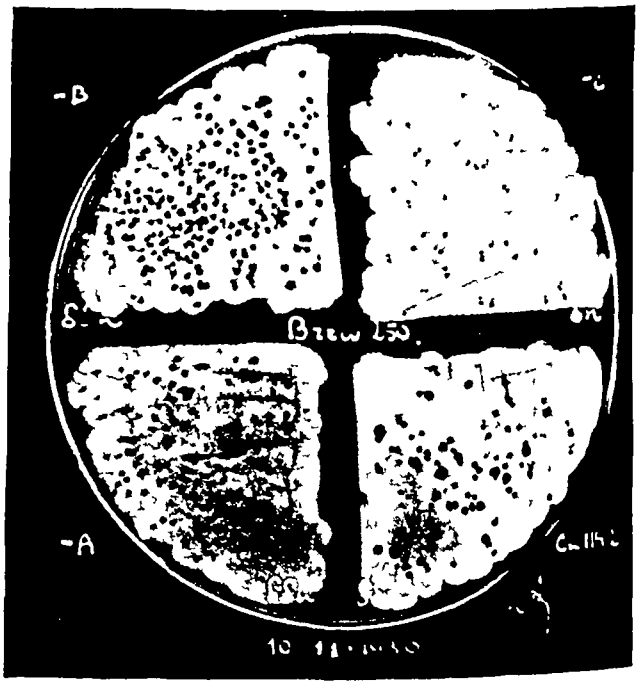

Fig 25 Separate counting of bacteriophage corpuscles belonging to different types in a mixture of bacteriophages, by means of 'One Type Sensitive' cultures The right lower quadrant plating of $Ch\phi ABC$ mixture with normal culture The same dilution of the mixture plated with 'oBs culture gives about ten times more clearings than with normal

TABLE II

	oTs				
	oAs	oBs	oCs	oDs	oEs
A	O				
B		O			
C			O		
D				O	
E					O
AB	O	O			
AC	O		O		
AD	O			O	
AE	O				O
BC		O	O		
CD			O	O	
DE				O	O
ABC	O	O	O		
ACE	O		O		O
BDE		O		O	O
ABCD	O	O	O	O	
BCDE		O	O	O	O
ABCDE	O	O	O	O	O

The ' OTS ' cultures can be used also as an additional test for ultra-purity of bacterial strains For this the tubes (*see* page 1149) with secondary growth are filtered and drops of the filtrates are deposited on the ' OTS ' spreadings If, for instance, the culture was contaminated with ChϕA, we will have —

	oAs	oBs	oCs	oDs	oEs
Filtrates (A) + A	O				
Filtrates (A) + B	O	O			
Filtrates (A) + C	O		O		
Culture alone	*				

* Here the clearing will appear only if the contaminating bacteriophage is easily separated from the culture under investigation

As I have mentioned in the section dealing with isolation of standard types of bacteriophage (*see* p 1140) we can meet a case where the ordinary plating of a mixture of bacteriophage will not reveal clearings of one of the types if it is present in small numbers, as it will be overgrown by the other bacteriophages present The ' OTS ' cultures sometimes can help us out of this difficulty

Taking again the example of choleraphage, suppose that the type-test indicated the presence in our filtrate of two types, ChϕB and ChϕE, while on the platings we are able to find the clearings of ChϕB only In this case let us take the ' oEs ' culture, spread it in the usual way on agar, incubate for one hour and then deposit a large drop of our B+E mixture on it Next day there will be a large clearing produced, of course, only by ChϕE, but containing all other bacteriophages If we now scrape the centre of this clearing with the loop, and wash it into an emulsion of normal cholera vibrio, and ' spread it immediately ' (direct, and dilutions like ' sl ' and ' bl ') we will have clearings of ChϕE predominating and it will be not difficult to pick them up and purify by repeated plating and isolation

The use of the ' OTS ' cultures was independently developed by Morison and Vardon (1930) in combination with the dilution method for the enumeration of corpuscles of different types of Chϕ present in a mixture of types For the reasons stated on page 1152 we abandoned this method altogether

Discussion

Many observations, though not pursued systematically enough to be published, make me certain that the principles of classification introduced here, and

true for cholera and dysentery bacteriophages, are equally applicable to any other bacteriophage But one must be reasonable and not attempt to apply these principles in cases where they are unsuitable In the suggested classification of bacteriophage I have arranged them in accordance with a system based on their action on bacteria Therefore this system depends first of all on the bacteria themselves possessing definitely known and stable characters In the case of cholera vibrios, which are to a considerable extent a homogeneous species, classification of bacteriophages presents a clear-cut system In the case of dysenteryphages we may experience difficulties on account of the considerable variations amongst the dysentery organisms themselves The clear-cut results obtained in the classification of bacteriophages by means of *B flexner* may appear irregular if for the same group of bacteriophages we use some atypical strain of dysentery bacilli In the case of *B coli*, where each strain presents some peculiarities, our typing of bacteriophages will be true in respect of the action of our bacteriophages perhaps only on that particular strain, and there might arise on this account a temptation to doubt the value of the suggested system But such a doubt does not seem logical For every system which introduces order in our knowledge, we must adopt a basis which in itself is not subject to variation, as no successful system can be based on an unstable foundation In the case of coli phages we cannot introduce a systematic classification based on their action on secondary growth, because *B coli* themselves cannot be classified To bacteriophage every strain is actually a nutrient medium on which it develops And as in bacteriology, when we want to arrange a group of bacteria in a system based, for example on the appearance of growth, we must use throughout a medium of the same composition, so in the study of bacteriophage we must use bacteria, which at least do not differ widely one from another If in the case of cholera the difference between the strains of vibrios is comparable to that between different batches of ordinary nutrient agar, in the case of *B coli* one strain may differ from another as much as ordinary agar differs from a potato slant

Fortunately, for classification of bacteriophages, besides their action on secondary growths another method is available which definitely supports the first the classification of bacteriophages by means of their antigenic value The use of bacteriophage anti-serum supports the first classification, proves its soundness, and, incidentally, can solve the difficulty when the first method leaves the possibility of doubt

It has been suggested to me that the types of bacteriophage represent only passing mutations of one and the same bacteriophage, i e, that the type characteristics are not fixed properties It is impossible in the present state of our technique and knowledge either to disprove or to confirm this definitely, but it is rather suggestive that during 4 years of daily experimentation first with three types of choleraphage, then with five, and with eight types

of dysenteryphage, submitting them to the influence of different conditions, I have never yet seen the transition of one type of bacteriophage into another Those characters of each race of bacteriophage which are not permanent type characteristics change freely, and each race shows considerable capacity for variation, but only within certain limits, never breaking outside the type to which it belongs

However, I am quite prepared to meet bacteriophage races which eventually may have to be called 'atypical', i e , which cannot be classified as belonging to one of the recognized types, whose type characteristics will not possess *all* the criteria of any one type The existence of such races of bacteriophage is not only possible and probable, but inevitable They will be exceptions confirming the rule

Our classifications of living beings are based on observation of their properties When sufficient evidence has been collected, we take into consideration only properties common to a considerable proportion of the organisms under study and arrange them in groups of common properties in accordance therewith, so arriving at rules of classification But every living being is subject to the constant process of adaptation and therefore of variation, and we are bound to meet individuals whose properties will not conform strictly to the rules of classification true for the majority As Beard (1931) rightly remarks, 'Every systematist is confronted with borderline types, the position of which in any scheme of classification is entirely arbitrary'

The suggested principle of classification and technique of determination of the types of bacteriophage provides us with a method giving clear-cut readings In fact, determination of the types of bacteriophage is more definite and unequivocal than determination of bacteria

It seems to me that whichever point of view on the nature of bacteriophage an investigator maintains, whether he accepts its living nature or not, he may with advantage accept the suggested classification for its evident practical value Whether there are different types of *Protobios bacteriophagus* or different lytic principles the important truth is that the difference exists and cannot be disregarded I have adduced sufficient evidence to warrant the conclusion that this difference is not accidental but falls into a definite system If we disregard this difference, throwing all bacteriophages into an indefinite mass as 'a bacteriophage', our work will lack system, our experiments will become random, and our conclusions will be lacking in certainty

Summary

A system of classification of bacteriophages, based on their reciprocal compensatory action on bacterial cultures, is suggested

A method, called Type-Test, is described giving the possibility (1) of determining the type of bacteriophage, (2) of ascertaining the purity of a bacteriophage culture, (3) of identifying the different types present in a mixture, and (4) of testing bacterial cultures for the presence of bacteriophage (Ultra-purity Test)

The use of bacterial cultures sensitive to one only of the types of bacteriophage is described

The significance and the value of the suggested classification is discussed

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STUDIES ON CHOLERA BACTERIOPHAGE

Part III

VIRULENCE AND DEVELOPMENT OF BACTERIOPHAGE

BY

IGOR N ASHESHOV, INNA ASHESHOV, M N LAHIRI AND S K CHATTERJI

(*From the Bacteriophage Inquiry, Indian Research Fund Association, Patna*)

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- Virulence of bacteriophage, its maintenance and methods of increasing it
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- The range of action of bacteriophage
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- Estimation of activity of bacteriophage suspension
- Summary

VIRULENCE is one of the most misused terms in biology in general and in connection with bacteriophage in particular. In general it is often confused with toxicity; the reference, for instance, to 'virulence' of cholera vibrio (which we have no means to determine), meaning its capability to kill an experimental animal, is not seldom met with. In connection with bacteriophage no one, with the exception of d'Herelle, has even given a definition of the term, and in publications which have appeared virulence has been confused with the activity of bacteriophage suspension, with the number of corpuscles, with lytic power, etc.

Before proceeding to discuss the virulence of bacteriophage we must first of all agree on a clear definition of the term. The definition which d'Herelle (1926) gives

in his book runs as follows 'the virulence is the power to develop within and at the expense of the host, and we consider the degree of virulence to be the higher as this development is more rapid', or, more shortly, further on 'the power of multiplication in a foreign host' It seems to me that the definition in case of bacteriophage can better be phrased as follows the virulence of bacteriophage is its power to develop within and at the expense of bacteria, causing their destruction The degree of virulence is considered the higher as multiplication and the destruction are the more rapid

However, it is necessary to admit that both definitions are far from being exhaustive The problem of virulence is so complex that we hardly can be expected to give a satisfactory definition in one or two sentences The definition given does not take into account the scope, range and extension of bacteriophage action For instance, we can have a bacteriophage multiplying very rapidly, quickly destroying bacteria, but attacking only a certain part of the culture and leaving a considerable number of bacterial cells present untouched Or we can have bacteriophage acting slowly but which can attack a much larger proportion of the culture or of varieties of the same species of bacteria In the first case we will have a quick lysis followed quickly by secondary growth, in the second a slower lysis and a later secondary growth Which of these bacteriophages is the more virulent? From this example it can be seen how difficult it is not only to formulate a satisfactory and complete definition of virulence but even to find a definite criterium of virulence At the present state of our knowledge on bacteriophage I consider it preferable not to complicate the problem but to be satisfied with the suggested definition of the virulence of bacteriophage as the power to destroy bacteria, depending on the rapidity of multiplication of the former at the expense of the latter

There is evidently considerable confusion as to whether this power belongs to the corpuscle or to the whole suspension of these corpuscles Both the above definitions imply that the virulence of bacteriophage is an individual intrinsic power belonging to the bacteriophage corpuscle, its property, one of its main characteristics The sum of this characteristic for all corpuscles present indicates the virulence of the race To attribute virulence to the whole suspension containing these corpuscles, in our point of view, is to confuse virulence of bacteriophage with activity of the suspension, which can be varied at will without affecting the actual virulence, by altering the number of corpuscles, for instance, by dilution This error seems to be quite common and has led to faulty comprehension and faulty methods of estimation of the virulence of bacteriophage

Two methods have been suggested for estimation of the virulence of bacteriophage One is to judge virulence by rapidity and permanency of lysis of bacterial culture, the other by enumeration of bacteriophage corpuscles present Both methods seem to me inadequate for the purpose

In the first instance we must take into consideration the complexity of the phenomenon of lysis On the one hand, since Serre (1929) proved the production of the lysins by bacteriophage, we must take into consideration that the liberated lysins can destroy free bacteria, i e, those cells which are not actually parasitized by bacteriophage corpuscles The fact that we do not yet know how far the destruction goes whether the free bacterial cells are only killed or also lysed (how far?) still more complicates our speculation on the point Approaching the phenomenon of lysis from such a point of view we may consider the races of bacteriophage producing the larger amounts of lysins not so much virulent as toxic We cannot call such races 'more lytic' because, as I have said we do not know how far the action of free lysins can go, and such term may introduce a further confusion into our observations In the case where free lysins do not produce complete lysis of the bacterial cell, but still render it unsuitable for bacteriophage to parasitize, we will have a paradoxical phenomenon, that a race of bacteriophage producing a larger quantity of free lysins* may produce less visible lysis of a culture in a test-tube The experimental evidence seems to support the last possibility I have noticed on many occasions that the races of bacteriophage which give clearings with large lytic (containing no bacteriophage) zones, usually produce a poorer lysis than bacteriophages giving clear-cut clearings without zones It means that the lysins can actually check the multiplication of bacteriophage On the other hand, since the lysins are destroying bacteria, our requirements for virulence—the rapidity of destruction, as mentioned in our definition of it—are fulfilled, and the race of bacteriophage giving only a partial lysis may still be quite virulent In view of such confusing evidence it seems to me that it would be better if we try and find some simpler and more definite way of judging the virulence of bacteriophage than that of estimation of the degree of lysis in a test-tube

Besides, up to the present, in estimating the virulence of bacteriophage by its lytic action, so far as I know, no one has yet taken into consideration the relative and absolute numbers of bacteriophage corpuscles and bacteria brought together It has always been definite quantities of bacteriophage suspension as opposed to definite number of corpuscles that have been used If we analyse the conditions, which arise from such a method we can easily see that virulence cannot be estimated in this way If we inoculate two identical emulsions with equal quantities of two suspensions of bacteriophage, the virulence of which we intend to compare, and observe that the first tube is lysed in 2 hours and the second in 3, we will conclude that the bacteriophage added to the second tube is less virulent than that added to the first But it may easily be that the first suspension of bacteriophage contained a thousand times more bacteriophage corpuscles than the second,

* There is a temptation to introduce the terms endo and exolysins, but there is already so much confusion about the process itself and its terminology that I prefer to refrain from using these

of lower virulence, the greater rapidity of the lysis being due only to the greater number of corpuscles It can be argued that the smaller number of corpuscles is in itself an indication of lower virulence Further on we will see that experiment proves that quite often the opposite is true, and therefore this argument cannot be accepted Moreover, so many conditions influence the final number of corpuscles that it cannot be accepted as evidence for any purpose Also, if we use such a method of estimation of virulence as described, we must arrive at the conclusion that by diluting a suspension of bacteriophage we will diminish its virulence, which is obviously wrong By using equal amounts of bacteriophage suspension against identical emulsions of bacteria we can compare only the activity of suspensions, but not the virulence of bacteriophage

The method of comparing the virulence of bacteriophage by enumeration of corpuscles present in two suspensions, even obtained under identical conditions, is also, in my view, quite erroneous Quite often we find in the literature on bacteriophage the statement that 'two bacteriophages were used, one of a low virulence, showing the presence of bacteriophage only in 10^{-5} dilution, the other very virulent, showing the presence of bacteriophage even in 10^{-10} dilution' This is a definite confusion of the number of corpuscles with their individual value d'Herelle (1926, p 149) states, 'If we inoculate a series of tubes containing identical suspensions of a susceptible bacterium with equal *quantities* (italics are mine—I N A) of bacteriophage suspensions derived from different sources, we will find, after a given time, the conditions throughout being the same, that the resulting bacteriophage suspensions will each contain a different number of corpuscles These numbers will be strictly proportional to the virulence of different bacteriophages' As we will see later, the resulting number of bacteriophage corpuscles will be different with different races of bacteriophage even if we inoculate the identical bacterial emulsions with equal *numbers* of bacteriophage corpuscles But in both cases the resulting number will not be proportional to the virulence and often it may even be inversely proportional to it

Indeed, what does the final number of corpuscles of bacteriophage depend on ?—(1) On the generation period of bacteriophage, which is an individual property of the bacteriophage corpuscles, the shorter it is the more generations will be produced in the same period of time, (2) on the number of corpusles produced from one corpuscle at each generation , the larger it is the greater will be the resulting total number of corpuscles, and (3) on the total number of bacterial cells available for propagation of bacteriophage, from the beginning until all have been destroyed or their further development arrested by the condition of the medium

On the other hand, what is the virulence characterized by ? By the two first of the factors just mentioned—generation period and the number of corpuscles produced at each generation And were it not for the last factor mentioned (the number of bacteria available for bacteriophage through the whole period of action)

Plate LVI

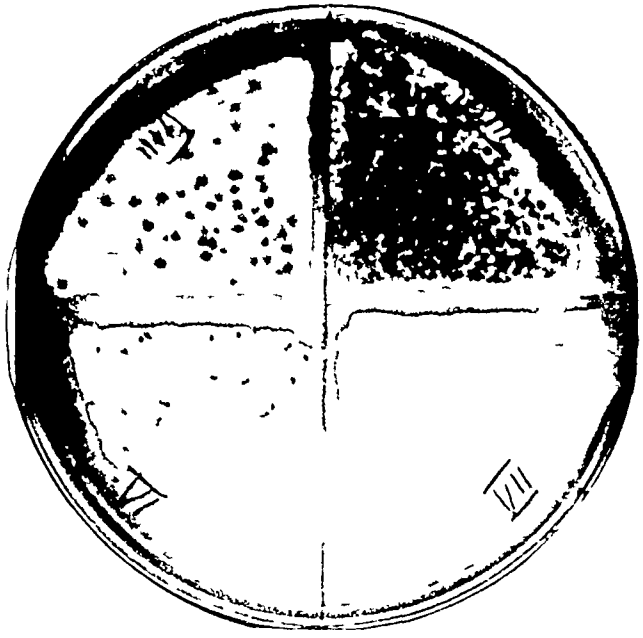


Fig 1 Different types of Shiga phage The most virulent is VI, the least—VII

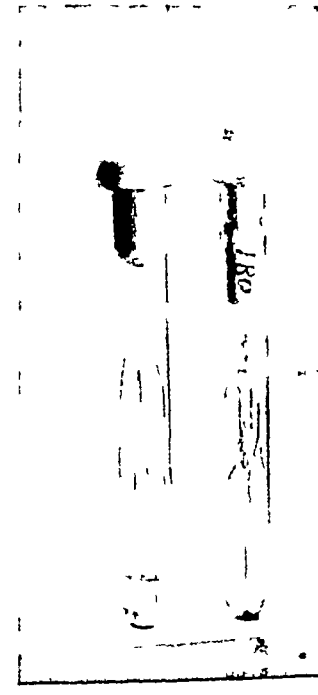


Fig 2 Arrangement for filling small number of ampoules

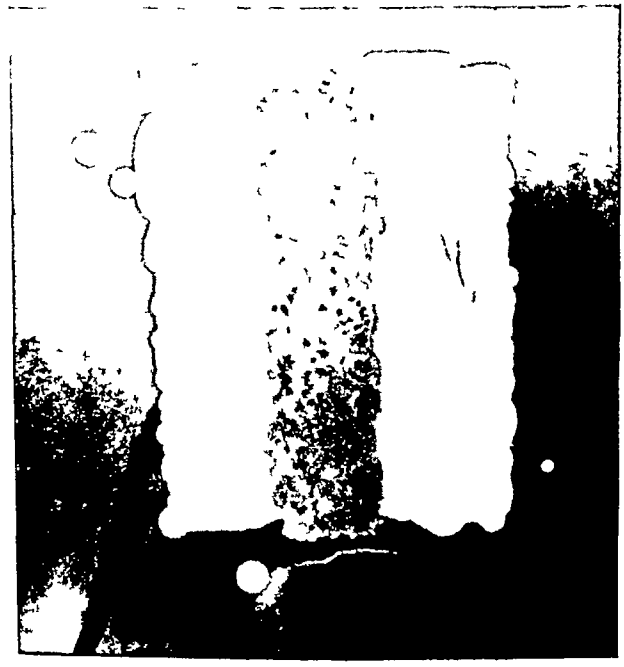


Fig 3 The 'secondary clearings' A heavy emulsion of Ch vibrio was spread on agar Over it ChϕB was deposited in a stripe The transparent part is secondary growth Some of the bacteriophages succeeded to overcome its resistance and produced the 'secondary clearings'

the final number of corpuscles obtained would serve as the true indicator of virulence But if we analyse what actually happens in the test-tube in which we grow bacteriophage to be examined for virulence, we will see that the final number of corpuscles does not depend on the virulence and cannot be directly proportionate to it

Let us take for example a very virulent bacteriophage and one of a medium virulence We inoculate two identical bacterial emulsions, with an equal number of each bacteriophage . The first bacteriophage will destroy all available bacteria, say in 2 hours, the second in six hours In which case will the total number of bacteria destroyed be the greater ? Undoubtedly in the second Therefore it can easily happen that, though the generation period of the weaker bacteriophage is longer and the number of corpuscles produced at each generation is smaller, the second bacteriophage developing considerably longer its resulting number of corpuscles can easily exceed that of the first (virulent) bacteriophage This is what actually often happens If we, for instance, inoculate two identical emulsions of rough-smooth cholera vibrio with equal number of ChϕA and ChϕC, in the majority of cases we will obtain a considerably larger number of corpuscles with the low virulent ChϕC (*See* Chart and Tables I and II—*vide infra*)

In general, taking into account the complexity of the course of the development of bacteriophage, influenced as it is by so many factors, it is easy to see that the final number of bacteriophage corpuscles under no condition can serve as an indication of the virulence of the bacteriophage corpuscle

Another suggestion by d'Herelle that the size of the clearings produced by bacteriophage is proportionate to the virulence is not supported by experiment On Plate LVI, fig 1, four different types of Shiga-phage are shown The most virulent of them is Type VI giving the smallest clearings, the least virulent is Type VII giving the largest clearings

What then will be the more correct approach to the solution of the problem of estimation of bacteriophage virulence ? From the definition of bacteriophage virulence we can conclude that it can be evaluated by the rapidity with which bacteria are destroyed and by the rapidity of the multiplication of bacteriophage Theoretically the first (the rate of destruction of bacteria) would seem to be sufficient if one bacteriophage destroys bacteria quicker than the other, evidently the first is the more virulent But there is one objection to such a conclusion I have already mentioned how complicated is the interaction bacterium-bacteriophage The action of the free lysins of bacteriophage on bacteria has as yet been very little studied By simply observing the rate at which bacteria are perishing, as in the case of observation of the lysis alone, we may be led to confuse the direct action of the living being bacteriophage and the action of a non-living substance—the lysin The first constitutes the virulence The second I have already compared with toxicity Moreover, the preliminary investigations into the technique

of estimation of the rate of destruction of bacteria showed that the problem is more difficult and complex than it seems The first problem which presents difficulties is the necessity of an instantaneous arrest of further action of bacteriophage at any given moment, to enable us to count the surviving bacteria The action of chemical substances (citrates, bile) proved to be unsatisfactory So also is the ordinary anti-phage serum containing bacterial anti-bodies, which influences bacteria to an unknown degree In short, this method is not yet available for practical application, though deserving further exhaustive study which is being carried out at present in this Laboratory

On the other hand, the estimation of the rate of multiplication presents very few practical difficulties The only possible objection is that we cannot yet say with absolute certainty that the rate of multiplication of bacteriophage is strictly proportional to the rate of destruction of bacteria We have, however, considerable evidence, theoretical as well as practical, that this is so

Virulence test

The rate of multiplication, that is to say the rapidity of the growth of bacteriophage, has already attracted the attention of other investigators Bail (1923) in particular used it for the differentiation of his *echte* and *unechte* (I would translate it as 'true and 'accidental') bacteriophages But no one to my knowledge has used the estimation of the true rate of multiplication as the indicator of virulence

I am of opinion that the true rate of multiplication can be estimated only if the observation is made during the period of unhindered free development of *all* bacteriophage corpuscles present The normal course of development of bacteriophage can be hindered (1) by insufficient number of bacteria, the effect being that not all the corpuscles of bacteriophage present can continue to multiply at the normal rate, and (2) if the bacteria themselves are hindered in their normal development, which usually occurs when either the number of bacteria exceeds a certain limit or when the medium is exhausted and is saturated with products of metabolism The only period then when we can make our observations will be during the first few hours after inoculation of bacteria with bacteriophage and provided the following conditions are observed —

(1) the period of observation must be as long as possible, limited, however, by the time when any of the inhibition factors enter into play,

(2) the number of bacteria introduced into the medium must be sufficiently large to provide 'food for *every* corpuscle of bacteriophage during the whole period of observation, but not too large, as that can prematurely inhibit the development of the bacteria themselves,

(3) the number of bacteriophage corpuscles inoculated must be as small as possible but sufficient to render easy the first estimation of their number

To satisfy these conditions the following technique has been worked out —

Short description a bacterial emulsion containing the optimal number of bacteria is inoculated with the optimal number of bacteriophage corpuscles An immediate plating is made to ascertain the actual number of corpuscles inoculated After the chosen time a convenient dilution of the contents of the test-tube *without filtration* is again plated Next day the number of clearings on both platings is counted and the number of corpuscles present at the two moments determined The number of corpuscles present at the moment of the second plating is divided by the number of corpuscles originally introduced This gives the number of corpuscles originated during the period of observation from one corpuscle and is called the rate of multiplication or indicator of virulence

The technique was worked out for cholera phages, but a few tests performed with other intestinal bacteriophages have suggested that it can be used without modification in their case too

Time of observation —This has to be chosen so as to enable us to compare bacteriophages of very different rates of multiplication The problem was solved almost of itself when we compared the most rapid ChϕA with the slowest ChϕC The rate of multiplication of the first, when using the chosen number of vibrios and bacteriophage corpuscles, has already started to slow down at the beginning of the third hour On the contrary ChϕC shows the first appreciable signs of development after an hour-and-a-half From Tables I and II (*see* page 1167) one would be inclined to conclude that for the quick acting bacteriophages like ChϕA it would be better to make the second plating after about 1 hour 30 minutes or 1 hour 45 minutes, while for ChϕC it would be better to plate the second time after about 2 hours 30 minutes This being so it was evident that a compromise is necessary and the time of the second plating was fixed at 2 hours, giving the possibility of comparing all varieties of bacteriophage

The number of vibrios used — Experience showed that 1×10^9 of vibrios for 20 c c of broth (5×10^8 per c c) gives quite constant and reliable results, though the number can be increased considerably without influencing the results, and therefore no particular accuracy is required in the determination of the number of vibrios taken We usually take 1 c c of 18 hours old peptone water culture, which ordinarily contains about 1×10^9 of vibrios per c c

The number of bacteriophage corpuscles inoculated —The lesser the number of corpuscles the more freedom they will have for development 6×10^2 per c c was chosen as the minimum giving the possibility of reliable enumeration at the first plating As our ' big loop ' takes about 0 025 c c on plating this amount immediately after bacteria and bacteriophage are added to the broth, we will obtain some 15 clearings—a number convenient to count and sufficient to ensure adequate accuracy The enumeration of bacteriophage corpuscles in the original suspension and the calculation of the amount of it necessary to be added to our experimental 20 c c of

broth to obtain 6×10^2 corpuscles per c c is very simple Suppose that on the usual plating of the original suspension we obtain with ' big of the small loop ' dilution 150 clearings This means that a ' big loop ' of the last dilution plated contains 150 corpuscles, while we want 15 Therefore, if we were using for dilution 10 c c, of broth, our bacteriophage suspension ought to have been diluted 150 15=10 times, but as we use 20 c c of broth in our test, the dilution must be only 5 times The procedure in this case will be as follows take one ' big loop ' of our original bacteriophage suspension in 10 c c of broth Transfer 1 c c of this dilution into another tube and add 4 c c of broth Transfer one ' small loop ' of this dilution into our 20 c c of broth On adding the required amount of bacteria plate the usual ' big loop '—it will give approximately 15 clearings, i e , 6×10^2 of corpuscles in 1 c c

Experiment shows that no particular accuracy in the number of corpuscles used is necessary—if even 12 times the number be taken, i e , $7\ 2\times10^3$, it does not influence the rate of multiplication It follows that, when we are comparing the virulence of two or several bacteriophages, there is no necessity to use exactly equal numbers of each A very wide approximation is sufficient

In Tables I and II and in the Chart (*vide infra*) we can see the results of using the suggested method The experiments are shown there somewhat *in extenso* to illustrate the further possibilities of the method For the routine examination of virulence it is sufficient to find out the rate at 2 hours

These tables also show, as I have stated, that the resulting number of corpuscles after 24 hours cannot be taken as an indicator of virulence This is particularly evident in the Chart

In performing this test we must take into consideration that each corpuscle of bacteriophage gives at the moment of division not two new corpuscles, as in the case of bacteria, but many (d'Herelle) Consequently, we cannot expect a regular flow of increase it is a matter of chance whether it will be gradual or in abrupt stages, depending on the number of corpuscles dividing at the same moment It follows that when we compare rapidly growing bacteriophages, and make our second plating after 2 hours, it can happen that one of them will be plated a few moments before an abrupt increase, while the other just a few moments after the abrupt increase The result will be a wrong conclusion that the second bacteriophage is more virulent Therefore I would advise not depending on the result of one test but on repeated examinations only Furthermore, when performing the test, it is advisable to make at least two platings from each tube at first from the first, then from the second, then again from the first and again from the second This will increase the accuracy of the test

Comparative value of the test —In estimating the value of the test we must first of all clearly understand that it has only a comparative and not an absolute value In estimating the action of bacteriophage on the culture we must take into

TABLE I

Comparative estimation of the rate of multiplication of five races of cholera phage, Type A

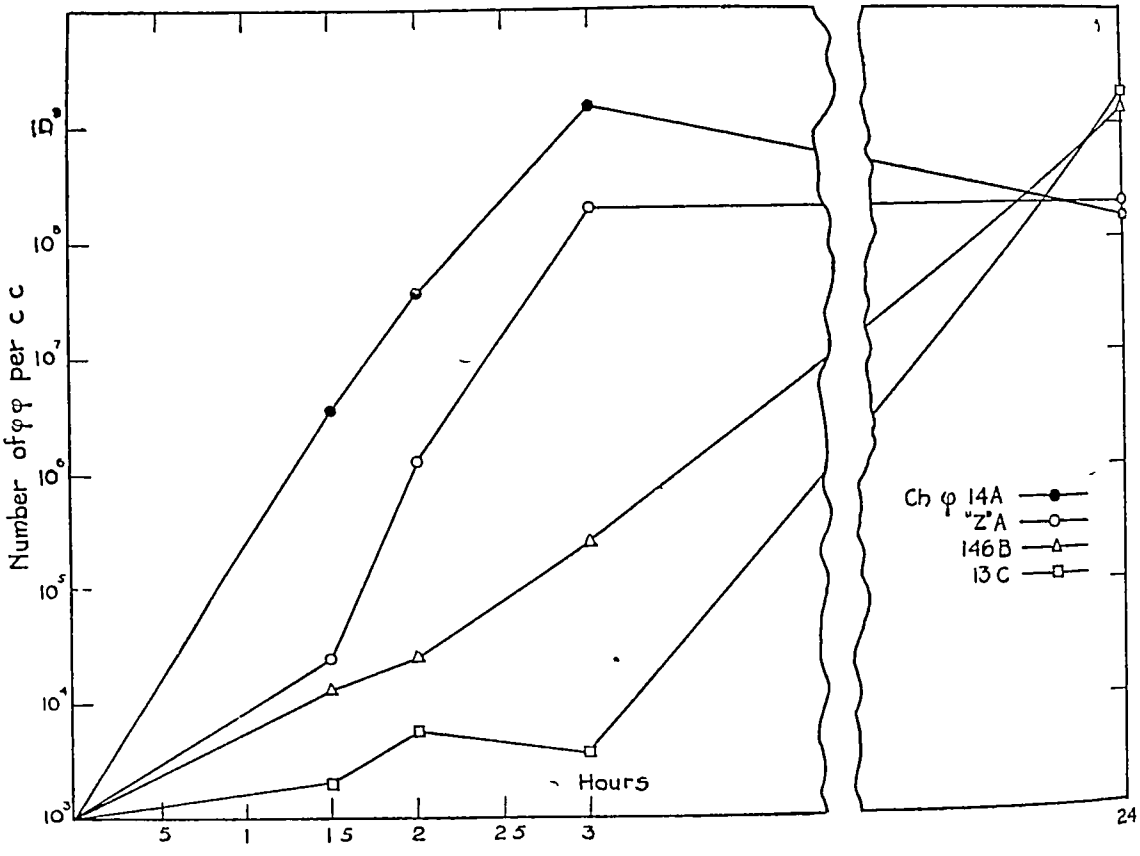
Ch φ	Number of corpuscles inoculated per 1 c c	Number of corpuscles after 1 hour 30 minutes	Rate	Number of corpuscles after 2 hours	Rate	Number of corpuscles after 3 hours	Rate	Number of corpuscles after 24 hours
14 A	4 8 × 10²	3 6 10	7 5 × 10⁴	2 8 10	5 8 10⁵	1 6 × 10⁹	3 3 × 10⁹	3 7 × 10⁸
14 α A	5 4 × 10	2 5 × 10	1 6 × 10⁴	1 8 × 10	3 3 10⁵	1 3 × 10⁹	2 4 × 10⁸	3 0 × 10⁸
14 β A	2 0 × 10	6 4 × 10⁶	3 2 × 10⁴	9 6 × 10⁷	4·8 × 10⁵	2 0 × 10⁹	1 0 × 10⁷	1 5 × 10⁸
71 A	3 0 × 10²	9 6 × 10³	3 2 × 10⁴	6 8 × 10	2 3 × 10⁵	1 6 × 10⁹	5 3 × 10⁹	2 5 × 10⁸
'Z' A	1 2 × 10	2 5 × 10⁻	2 0 × 10³	1 8 × 10⁹	1·5 × 10¹	1 2 × 10⁷	1 0 × 10	8 0 × 10⁸

TABLE II

Comparative estimation of the rate of multiplication of four types of cholera phage on laboratory strain Ch 1573 *isolated* 6 *months previously and on freshly isolated Ch* 1481 *Cal* (*first subculture from* ¼ *per cent peptone water*)

Ch φ	Tried on cholera vibrio	Number of corpuscles inoculated per 1 c c	Number of corpuscles after 2 hours	Rate	Number of corpuscles after 24 hours
14A	1573	1 6 × 10²	5 0 × 10⁵	3·1 × 10⁶	1 2 × 10⁹
	1484	1 9 × 10	1 0 × 10⁷	5·2 × 10⁶	2 1 × 10⁹
146B	1573	5 2 × 10²	2 5 × 10	5 0 × 10²	2 7 × 10⁹
	1484	6 4 × 10	5 0 × 10⁵	8 0 × 10²	2 9 × 10⁹
13C	1573	2 2 × 10	6 4 × 10³	2·9 × 10¹	3 4 × 10⁹
	1484	1 2 × 10	9 2 × 10⁷	0 8 × 10¹	1 2 × 10⁹
D	1573	1 0 × 10	2 4 × 10⁴	2·4 × 10²	3 4 × 10⁹
	1484	2 2 × 10	4 0 × 10⁴	1 8 × 10²	1 8 × 10⁹

consideration that we have to deal with more than two variables—bacteria, bacteriophage, media, amongst others—all very sensitive to any change of environment and influencing each other



Environment can influence bacteriophage in two ways directly and through bacteria, though it is impossible to separate these ways, and what influences bacteriophage influences at the same time bacteria as well The factors which can influence bacteriophage development directly are temperature and air supply, while any factor which brings alteration in the growth of the bacterial culture which serves for the multiplication of bacteriophage, immediately affects also the development of the latter Very important is the state of the culture and the phase of S-R dissociation, which in their turn are functions of the age and method of cultivation

As all these factors change constantly and both bacteria and bacteriophage are very sensitive to their influence, it is evident that it is next to impossible to repeat any of our observations under identical conditions It follows that a test performed to-day cannot be compared with one performed yesterday, as we never

can guarantee that all the conditions are in both cases the same The suggested test has therefore a purely comparative value Speaking strictly we can compare the virulence of two or many bacteriophages only when testing them simultaneously However, in practice this is true only when we are comparing bacteriophages with relatively slight differences in virulence If the difference is considerable, as for instance in the case of Ch$\bar{\phi}$184A and Chϕ'A' (Table I, page 1167) the influence of the change in conditions can be disregarded as comparatively insignificant

The question arises, of which bacteriophages can we compare the virulence? Can a comparison be made of the virulence of different types? I am of opinion that strictly speaking we can compare the results of the test only if made with bacteriophages belonging to the *same* type, or at least to the same Burnet's group ('smooth', 'smooth-rough' and 'rough' bacteriophages) This statement may be supported by taking as an example an extreme case—that of ChϕA and ChϕE The development of these two types is essentially different the first uses only the smooth elements of the culture, the second only the rough Even if we make our comparative test using the same S-R strain of vibrios, it amounts to the use of two different cultures—S and R—and, strictly speaking, the results obtained under the conditions ought not to be compared It would be like comparing the virulence of *Meningococcus* with that of *C diphtheriæ*

Practically, we are bound to speculate on the comparative virulence of different types if only to understand their different rôles in the destruction of bacteria

From the results of the suggested test, even from the few data shown in Tables I and II, it is evident that it is impossible to compare the virulence of mixtures In this case we can only find the rate of multiplication of single types which enter into the composition of the mixture The mixture can be investigated only from the point of view of its activity, which will be discussed later

The choice of the culture for virulence test—From what has been mentioned about the influence of the state of the culture on the multiplication of bacteriophage, it may be inferred that some cultures may be more suitable for our purpose than others As the test is designed for various purposes we must expect that in different cases we will benefit by choosing different cultures to suit our particular purpose This question has not yet been completely studied and I am able to give only general indications It seems to me that when it is a question of choosing the best bacteriophage for practical application, we must use for the test only freshly isolated cultures, as little attenuated as possible by our artificial methods of cultivation This will give us a better idea of how the bacteriophages under examination will actually act in nature

On the contrary, for basic research in the study of bacteriophage characteristics in general, it seems to me better to use a laboratory-stabilized culture Such a culture is less liable to undergo appreciable changes under the influence of laboratory conditions This will give us the possibility of a better comparison of

the results obtained at different times 'bacteria factor' will be more permanent and constant However, I would advise always verifying the results obtained with laboratory cultures on the cultures which we consider more natural

APPLICATION OF THE VIRULENCE TEST

Though this test actually gives only an estimation of the comparative rate of multiplication of bacteriophages, it can be used for many purposes At present I will mention only the following —

1 Choice of bacteriophage of required virulence

2 Choice of the most suitable strain of bacteria for propagation of bacteriophage

3 Study of the influence of environment on the virulence of bacteriophage and of the methods for increasing the virulence of bacteriophage

4 Study of the methods for increasing the range of action of bacteriophage

5 Study of the influence of one type of bacteriophage on the development of another

1 *Choice of a bacteriophage of required virulence*

In the majority of cases it is a question of choosing the most virulent race for practical application As I have mentioned, in this case is it necessary to use freshly isolated bacterial cultures We usually proceed in the following way, which has become our routine —

For each bacteriophage two cultures are used the 'best' and the 'worst', determined as described in the following paragraph under (*a*) If the results of the test are inconclusive, i e , if the difference between the races of bacteriophages examined is not sufficiently pronounced, the test is repeated until a more conclusive impression is gained When the next lot of bacteriophages is examined, the best race of bacteriophage as determined in the previous test is included for comparison If a bacteriophage is found which acts better on the 'worst' culture, it is taken for further detailed study, and if sufficient reasons are found, it is included in our mixture for practical application In this way we hope to improve further and continue to improve constantly our mixture of bacteriophages used for prevention and treatment The test gives us the possibility of making this improvement rationally and not at random

2 *Choice of the most suitable strains for cultivation of bacteriophage*

Our ideas, before the introduction of the virulence test, as to the suitability of a culture for propagation of bacteriophage were rather vague we had no definite criteria for judgment We tried to judge by different standards which often proved to be irrelevant So, for instance, one was tempted to use for the propagation of bacteriophage the most lysable culture—the culture which either was easily and completely lysed, or which showed less of the secondary growth afterwards

The virulence test revealed that the lysability or the absence of secondary growth do not run proportionally to the capability of the culture to increase or maintain the virulence of bacteriophage

Again, the culture which, *ceteris paribus* gives a larger resulting number of bacteriophages may be useful, but only the virulence test will show whether in increasing the number of corpuscles in the resulting filtrate we do not diminish the virulence of the corpuscle

We also tried, before the test was introduced to judge the suitability of a bacterial strain by the number of clearing produced by the same quantity of the same bacteriophage suspension on agar platings We thought that if we plated the same dilution of a bacteriophage suspension using simultaneously several cultures, that which revealed the largest number of clearings would be the most suitable for the propagation of bacteriophage The test revealed that the opposite is often true Evidently, the culture which allows more corpuscles to develop clearings, gives to less virulent corpuscles, which would be eliminated in case of another culture, a possibility of multiplying Therefore, when using a culture giving fewer clearings with the same dilution of bacteriophage suspension, we help in the natural selection of more virulent individuals—though the question whether the virulence of these selected corpuscles on this particular culture will be maintained still remains open until confirmed or negatived by the virulence test

The test performed with the object of choosing a suitable culture can provide us with information on three points —

(*a*) On which culture does the bacteriophage grow quickest, i e , on which culture does the bacteriophage show the highest rate of multiplication ? This does not give us much information as to the suitability of the culture, indicating only as it does which strain of bacteria is the most sensitive to our bacteriophage

(*b*) On which culture does our bacteriophage give the largest resulting number of corpuscles ? This is tested by additional plating after 24 hours This information can help us in the final selection of a strain when we have ascertained the suitability by another test

(*c*) Which strain does actually help either in maintenance or in increase of virulence of our bacteriophage ? This is the crucial test and is made in the following way —

The usual passages of our bacteriophage are made on all the strains of bacteria under examination After the first and after the fifth passage on these strains the bacteriophage suspensions obtained are submitted to the test along with the original non-transferred suspension using the strains of bacteria most and least sensitive determined as under (*a*)

The best strain for propagation will be of course one which at least maintains, if not increases the virulence, and which at the same time gives the largest number of resulting corpuscles

By means of the virulence test we can now in a rational manner choose for each type, for each race of bacteriophage used, the most suitable strain of bacteria

Before the introduction of the virulence test I maintained the opinion that the bacteriophage mixture used for practical application should contain only one representative of each available type of bacteriophage—the best of each seemed to be sufficient The test performed as under (*a*) seems to indicate that it is better to use also different races of the same type as some races are more active against one group of vibrios, some against another By using different representatives of one and the same type, maintaining them on their best strains, we may improve the polyvirulence of our mixture

3 *Study of the influence of environment on the virulence of bacteriophage and of the methods for increasing virulence in general*

The virulence test has a very wide scope in the study of the influence of the conditions of cultivation on the quality of bacteriophage and of methods for its improvement It gives us the possibility of examining the influence of media, temperature, relative numbers of bacteria and bacteriophage used, of hyperaerobiosis, etc For this it is sufficient to apply the particular conditions to a bacteriophage during a number of passages and then to make the parallel virulence test of the bacteriophage so influenced and the original one The rate of multiplication will show whether the method of cultivation used brings any improvement in the virulence or not

4 *Study on the range of action of bacteriophage*

One who has worked with bacteriophage will know that races of bacteriophage belonging to the same type can differ not only in the rapidity of the destruction of bacteria but also in completeness and permanency of this destruction With some races the secondary resistant growth appears very soon after the lysis is complete, with others its appearance is protracted In the majority of cases of the last instance it means that the particular race of bacteriophage destroys more of the varieties of bacterial cells present in this culture, that the range of action of this bacteriophage is larger

The virulence test performed in the usual way does not give us any information as to the range of action of the bacteriophages examined However, a slight modification—only in the choice of the culture on which the virulence is tried—can assist us in the study of the range of action also

Sertić and Gough (1930) showed that the secondary resistant growth appearing after the action of bacteriophage is not homogeneous but consists of elements of different degrees of resistance They also devised a method in which by adaptation of bacteriophage first to the least resistant (r^1) bacteria, then to bacteria more and more resistant (r^2, r^3, r^4), they succeeded in gradually increasing the

range of action of bacteriophage resulting in more complete lysis of the original culture and in later appearance of the secondary growth

If now we take for performing our test not the normal culture but one of the resistant (r^1, r^2, r^3), we can, judging by the rate of multiplication of our bacteriophage on these components, obtain an idea of its range or of increase of this range The test in this modification can be, of course, applied not only to Sertić's method but to the others which have the same purpose of increasing the range of action of bacteriophage

5 *Study on the influence of one type of bacteriophage on the development of another*

This, a very important page of the history of bacteriophage life, is as yet very little studied, mainly because no sufficiently reliable methods have been worked out for adequate observations Now, combining the anti-serum method with the virulence test, we are able to follow with considerable precision the development of each type of bacteriophage separately while they are growing together, and to study the influence of one on the other

This presents no difficulty and requires no anti-phage serum when we are observing the development of a quick growing bacteriophage like Ch ϕ A in the presence of a slow growing bacteriophage like Ch ϕ C the high rate of multiplication of the former gives us the possibility of eliminating other types from our platings by simple dilution On the other hand, when we want to study the development of a slowly growing bacteriophage in the presence of a ' high rate ' bacteriophage, we must revert to the use of specific anti-phage serum which will inhibit the appearance of the clearings of ' high rate ' bacteriophage and give the possibility of using for plating adequate dilutions showing the clearings of ' low rate ' bacteriophage only

Virulence of bacteriophage, its maintenance and methods of increasing it

Virulence of bacteriophage and bacterial cultures —The practice of many years has shown me that the best way to increase or maintain the virulence of a bacteriophage race is to use for its propagation an adequate bacterial culture It is the same as with bacteria where some media help to maintain or increase the virulence of a strain, some attenuate it For bacteriophage we also have ' good ' and ' bad ' cultures On the first its virulence improves or is maintained, on the second it deteriorates

Therefore the virulence of the races of bacteriophage which we maintain in the laboratory depends largely on the proper choice and on the proper maintenance of the bacterial cultures which serve for the propagation of bacteriophage

The choice of the culture is made without difficulty by means of the virulence test as described under (2) (*see* page 1171), which avoids any arbitrary element in

our choice On the other hand the preservation of bacterial cultures without altering their natural properties presents considerable difficulties, while it is of paramount importance that we should use for bacteriophage propagation bacterial cultures as little altered by our laboratory methods as possible

We have tested many different methods of cultivation and maintenance of bacterial cultures for the purpose of finding one which will alter the culture the least In brief our conclusions are as follows —

All the usual methods of cultivation on ordinary solid and fluid media alter bacteria very quickly—after one or two weeks of such cultivation they are already 'laboratory strains', which, if used for propagation of bacteriophage, tend to deteriorate the latter also

Quick transfers (every four to six hours) in liquid media tend to prevent the S-R dissociation On the contrary, if we leave the culture in a liquid medium longer, the R forms start to appear very quickly For instance, cholera vibrio culture, if left in a liquid medium for forty-eight hours or submitted to two to four transfers in a liquid medium at twenty-four hours intervals, will contain a very large proportion of R elements

Cultivation or maintenance of the culture on agar, provided it is slightly acid or neutral, prevents the S-R dissociation, while an alkaline medium hastens it All the usual bacteria produce on ordinary solid media a considerable amount of alkali, so that within one week the whole slant is already strongly alkaline and the number of R forms in the culture increases For this reason, I advise (*a*) using the agar of pH 6 6 to 6 8, (*b*) adding to it an indicator such as cresol red or thymol blue to enable the reaction of the medium to be judged at a glance at any moment of cultivation, and to transfer the culture in time, before too strong an alkaline reaction starts to act, and (*c*) seeding the slant not in a continuous layer or line but in one or two spots only, so that medium will change its reaction more slowly

Nevertheless all these precautions are not sufficient to preserve bacteria in their natural state How rapidly and easily these changes appear can be judged by the lysis produced by bacteriophage as described further on

After numerous trials we have found that only the following method gives us the possibility of keeping a culture of such a sensitive organism as *V cholera* in a state very little altered for about a fortnight or even a month The stool of a cholera patient is plated directly on bile agar without preliminary enrichment in peptone water Well-isolated colonies are selected and picked up into 1 per cent peptone water As soon as the first traces of growth appear, one c c of this culture is transferred into eight to ten c c of $\frac{1}{4}$ per cent Difco peptone water and immediately sealed in 1 c c ampoules

The easiest way to fill small ampoules is the following Some eight of them are assembled in a large test-tube, open end down. About ten c c of $\frac{1}{4}$ per

cent peptone water is added and the whole sterilized at 110°C for 15 minutes (Plate LVI, fig 2) One c c of the culture, as described, is added and the whole tube put into a Martin flask which is closed with an india-rubber stopper The air is exhausted as far as possible and then admitted back through the air filter, the capsules filling on re-admission of air (The exhaustion is better maintained for a while to drive out the air dissolved in the peptone water, as this seems to assist in better preservation of the natural properties of the culture) The ampoules are sealed in the usual way and preserved in a refrigerator

For use, one whole ampoule is added to 25 c c of peptone water After a short incubation—not more than 8 hours, better 6—during which the tube is frequently energetically shaken to provide a rich supply of air, one c c of this culture is added to 10 to 20 c c of broth and inoculated with the bacteriophage to be propagated

How far and how quickly ordinary methods of cultivation alter a culture and on the other hand how far the suggested method is better, can easily be ascertained by the following experiment From the same tube wherefrom 1 c c of the culture was transferred into ½ per cent peptone water, seed an agar slant Next day two peptone water tubes are seeded, one from this agar slant, the other from one of the ampoules After overnight incubation (16 to 18 hours) both cultures are tested for lysability Both tubes are brought to the same turbidity by the addition of peptone water to the more turbid One c c of each culture is added to 10 c c of peptone water and inoculated with one drop of quick-acting bacteriophage (like ChϕA) The culture prepared directly from the ampoule will be lysed more quickly and more completely If this experiment is repeated with the same culture after a fortnight's preservation, the difference will be more striking

The choosing of the most suitable culture of vibrios for propagation of different types of Chϕ by means of the virulence test has shown that it is hardly possible to find a strain which will be the best for all types Even bacteriophages belonging to the same 'smooth-rough' group of Burnet, as for instance ChϕB and ChϕC, require different cultures for their best maintenance, to say nothing of bacteriophages belonging to the different groups, the extreme example being ChϕA and ChϕE We expected this as bacteriophages have a preference for different phases of dissociation of vibrios Though there is evidence that more subtle differences in the properties of a culture than that mentioned also influence its suitability for different races of bacteriophage, the state of dissociation is most prominent Information on this point will help us to understand and better to interpret many other phenomena connected with the life of bacteriophage

We have examined all the known methods which help us in judging the state of S-R dissociation of a culture morphology of colonies, growth in liquid media, influence of NaCl concentration, type of agglutination, etc, and came to the

conclusion that the easiest and at the same time an entirely satisfactory method for work with bacteriophage, is the method suggested by P Bruce White of using the Millon reaction It is very sensitive (even too sensitive if used as P Bruce White advises it), and gives quick and trustworthy results I have slightly modified the method, making it more suitable for bacteriophage work

Millon's reagent —

Metallic mercury	1 part by weight
Nitric acid 36°B	2 parts by weight

When the mercury is being dissolved in the nitric acid, dense fumes of nitrous oxide are given off It is better to do this in a fume chamber or in the open air After the dissolution is complete, the green liquid obtained is diluted with two parts of water *The reagent is then poured into a large photographic dish and aerated for about 24 hours, until almost all traces of smell have disappeared*

The procedure in making the test A loopful of 24 hours' growth of microorganisms on agar is emulsified in 2 c c of tap-water, 0 2 c c of Millon's reagent is added, the tube is left for about one minute, and then heated nearly to boiling point The heat is maintained for about one minute more, but without boiling

Three phenomena are observed —

1 *Flocculation* —With pure rough culture this appears even without heating, the floccuh slowly falling to the bottom On heating, the floccuh 'coagulate', partly floating on the froth, partly falling to the bottom, clearing the liquid more or less completely The more complete the coagulation and the clearer the liquid the more rough is the culture

2 '*Creeping*' —After heating, it will be observed that a film, more or less granular, is 'creeping' up along the wall of the test-tube above the surface of the liquid It consists of coagulated vibrios which float on the surface of a thin layer of the liquid adhering to the wall The more marked the phenomenon the more rough is the culture

3 *Brick coloration* —With non-aerated reagent and when kept in a tightly stoppered bottle, the more rough is the culture the more intensive is the brick coloration of the sediment that appears But as every culture always contains some other organic matter besides bacteria, and as every cholera culture, however smooth, contains some of the rough elements, this coloration always appears and is somewhat deceptive It was found that if Millon's reagent is aerated as advised it no longer produces this coloration For reading the results of this test the first two phenomena—flocculation and 'creeping'—are sufficient to judge the state of dissociation of a culture

An emulsion of smooth culture remains evenly turbid, that of a rough completely coagulated, leaving the fluid clear In between these two extremes the reaction can show all intermediate degrees, indicating the predominance of smooth or rough

elements For practical purposes it is quite sufficient to distinguish three degrees S, S-R and R If more exact description is wanted, the symbols sR and Si could be used indicating the presence of small amounts of smooth or rough elements respectively

As I have mentioned, for some purposes in bacteriophage study it is preferable to use a laboratory stabilized culture It often makes the carrying out of experiments easier, and gives results more easy to compare as such a culture is less liable to daily changes than a freshly isolated one The culture is chosen by means of the virulence test A further improvement in this culture can be brought about in the following way by gradual selection —

The best culture is spread on agar and some 5 colonies are picked up and subcultured They in their turn are tried by means of the virulence test, along with the original culture If one of the so obtained strains gives better results, it is in its turn spread, some colonies are subcultured and tried,—and so on Often in this way one succeeds in improving the virulence of bacteriophage for laboratory cultures Whether this procedure improves it for the natural, freshly isolated cultures can be proved only by experiment, and more often than not such a bacteriophage of exalted virulence for laboratory cultures loses its virulence for the natural cultures

It goes without saying that by the same method of gradual selection we can obtain also particularly lysable cultures or cultures on which a bacteriophage shows the largest rate of multiplication

To induce some particular property in a bacteriophage, e g , to enlarge its range of action, to improve its stability, etc , it is often inevitable that we should submit it to prolonged propagation under laboratory conditions This, of course, though improving the race of bacteriophage from certain points of view, will 'denature' it, and I would not recommend using such a bacteriophage for practical application without previously restoring its natural character This can be achieved by passages of our 'laboratory-brought-up' races of bacteriophage through cholera patients We did this in the following way —

First we ascertained that a race of bacteriophage 'educated' in the laboratory to a satisfactory standard does not lose the acquired properties even on prolonged passages through human beings It was logical to suppose, and was proved on many occasions, that the re-isolated bacteriophage would vary considerably in its individual virulence This fact is easily explained by the existence of the 'good' and 'bad' strains of cholera vibrio mentioned above Indeed, if a patient has an attack of cholera due to a strain of a vibrio incapable of maintaining the virulence of bacteriophage, the re-isolated bacteriophage will be of a lower virulence than the one given On the other hand in the case of an attack of cholera due to a 'good' vibrio, we might expect to re-isolate a bacteriophage of even improved virulence

The technique of the human passages was worked out as follows —

When we started our experiments there were very few patients in the hospital, new cases arriving after intervals of several days One of the patients was given a pure culture of one type of bacteriophage During the following days stool specimens were regularly collected Races of bacteriophage present in the filtrates of the stool were passed only once, preferably on the vibrio isolated from the same patient, and then compared for their individual virulence The re-isolated race, which proved to be the best, provided it was at least as virulent as the one given, was administered to the next patient This procedure was repeated many times After that, further administration of the bacteriophage was stopped altogether, but it was found that the newly admitted patients, in spite of this cessation, soon after their arrival—sometimes even after 8 hours and certainly the next day—were found to be infected with the bacteriophage introduced previously by us into the hospital This was observed even when a patient was admitted to the hospital when there were no other cases to infect him with bacteriophage, the last case of cholera having been discharged some days previously Evidently everything in the hospital—utensils, linen, hands of attendants—was harbouring the bacteriophage which easily contaminated the newcomer In that way, in contaminating the hospital itself with the bacteriophage, we succeeded in effecting the most natural passages of bacteriophage The stools from these patients, naturally infected with bacteriophage, were regularly collected and examined for the presence of bacteriophage After ascertaining, by testing for example their polyvirulence, that the bacteriophages present were the same as introduced by us, their virulence was compared amongst themselves and with that of the original bacteriophage A part of the direct filtrate of the stools containing the best races was preserved in sealed ampoules (*see* Plate LVI, fig 2) for further use, and a part was subcultured once only on the vibrio isolated from the same patient Only this first transfer was used for the production of bacteriophage for field use In that way the requirements of bacteriophage being (*a*) 'natural', (*b*) virulent and (*c*) with wide range of action, were satisfied

Here it is not out of place to mention the significance of the existence of 'good' and 'bad' strains of bacteria for the propagation of bacteriophage from the epidemiological point of view It may explain why some epidemics quickly subside while others persist for a long time It is quite possible that in the first instance the epidemic is due to a 'good' strain and bacteriophage propagating on these vibrios rapidly gains virulence and prevents further advance of the epidemic, while in the second case the epidemic may be due to a 'bad' strain, and even if we import there a virulent bacteriophage, it may, propagating on these 'bad' strains, quickly lose its virulence and its effect will be greatly diminished

Amongst the methods of increasing the virulence of bacteriophage, the following are worth trying —

1 Sertić (1929) showed that amongst a great number of bacteriophage corpuscles of the usual virulence there are often present a few of a higher virulence If we transfer only small amounts of bacteriophage each time, we may eliminate these most important individuals On the contrary, if we use a relatively large number of corpuscles and a small number of bacteria only the more virulent bacteriophages will have the opportunity to multiply We found the following method the best to adopt in using this principle —

To 20 c c of broth is added one drop of bacterial culture when dealing with quick acting bacteriophage, or one ' small loop ' in the case of a slow one, and it is incubated with 1 or even up to 5 c c of bacteriophage suspension After 3 hours in the case of a quick bacteriophage (such as ChϕA) and up to 6 hours in the case of slow bacteriophage (such as ChϕC) the addition of the culture is repeated If possible the addition of the culture is repeated a third time Next day the whole is filtered and a transfer is executed in the same way After some 5 to 10 such passages the increase of virulence can be ascertained by the virulence test, using for comparison the original race, transferred in broth in the ordinary way, along with the experimental tube

Two other methods were suggested to me by d'Herelle's statement that bacteriophages fix themselves to susceptible bacteria with rapidity proportional to their virulence

For both methods the time of fixation of the bacteriophage under examination is first of all ascertained For this, to 15 c c of broth are added 5 c c of bacterial emulsion and 1 c c of bacteriophage suspension Some 5 or 6 identical tubes are prepared simultaneously with a control, which consists of 20 c c of broth and 1 c c of bacteriophage suspension The control tube and the one with emulsion are filtered immediately, the remaining tubes at intervals of 5 minutes All the filtrates are then plated and the number of corpuscles counted The time required to absorb about $\frac{1}{4}$ of the bacteriophage corpuscles is noted

(*a*) *Centrifugalization method* —A normal emulsion of bacteria—1 c c in 20 c c of broth in a centrifuge tube—is inoculated with 1 c c of bacteriophage suspension After the time required for about $\frac{1}{4}$ of the corpuscles to be absorbed the tube is centrifugalized at a high speed until the supernatant liquid is clear The liquid is rejected, the bacteria resuspended in broth, and the bacteriophage fixed to them is left to develop overnight After some 5 to 10 such consecutive treatments the virulence is investigated by means of the virulence test

(*b*) *Filtration method* —The same emulsion inoculated with bacteriophage as in the case of (*a*) is prepared and after the required time filtered through a collodion membrane of about '20 sec ' permeability (Asheshov, 1933) and repeatedly washed

through with saline or broth, then resuspended in broth and the fixed bacteriophages are left to develop The further procedure is as under (*a*)

In both of these methods we eliminate by filtration or centrifugalization the less virulent bacteriophages, using for further propagation only bacteriophages which have fixed themselves early and are expected to be more virulent In this way we achieve a selection of the more virulent bacteriophages

Environment and virulence of bacteriophage

As I have already mentioned, physical and chemical conditions influence the development and properties of bacteriophage considerably Amongst the factors influencing bacteriophage directly or through the change in bacteria are composition of medium, temperature and aeration

Composition of medium —Generally speaking, the better the medium is for the development of bacteria the better it is for the development of bacteriophage as well But this is not so in every case

We spent considerable time trying to work out a cheap meat-free medium to be used for preparation of bacteriophage in large quantities for practical application Using the same methods as for meat media—digestion with pepsin-pancreatin and with papain, we tried in 1929-1930 to prepare one from vegetable substances rich in protein, such as some varieties of 'dhal'* and from casein We succeeded in obtaining media of good nutrient properties and giving quite rich growth of bacteria At the same time we found that the development of bacteriophage in these media is far from being as good as in papain meat broth and therefore their further use was abandoned

As another example of the influence of the composition of media on the development of bacteriophage may be cited the case of sugar media The addition of even a small amount of glucose considerably enhances bacterial growth It might be expected that this would also stimulate the development of bacteriophage provided that the reaction of the medium is always kept at optimum However, it is not always the case and a most interesting fact is that different types of bacteriophage behave differently in this respect So, for instance, the addition of 1 per cent of glucose increases considerably the rate of multiplication and the resulting number of ChϕA corpuscles, while the rate of multiplication and the number of ChϕB and ChϕC is distinctly diminished We use this property of glucose to increase the relative and absolute number of ChϕA in our mixture of Ch$\phi\phi$ issued for practical purposes

Temperature of incubation —Many observers have already stated that it is of advantage to cultivate bacteriophage at a lower temperature than 37°C We have

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* Indian pulse The grains are husked and reduced to coarse flour One of the richest in protein (23 6 per cent) is the variety called 'boot' or 'chana' (crushed gram)

also observed that sometimes a bacteriophage gains in virulence if cultivated at room temperature

Aeration of bacteriophage cultures—d'Herelle in his book states that a free air supply is essential for the development of bacteriophage and that in hyperaerobic conditions bacteriophage lyses quicker and soon gains in virulence

We investigated the question in the following ways —

1 Influence of aeration on the rapidity of development of bacteriophage Broth was distributed by 18 c c in Legroux tube and in ordinary 8 inches×1 inch tube, giving in the first a layer of liquid 0 5 cm and in the second 5 5 cm high To these two tubes were added the same amount of vibrio culture and of bacteriophage suspension as for the virulence test The platings were made at once and after 2 hours

Our conclusions are that increased aeration increases the rapidity of development of bacteriophage very considerably—up to 20 times in case of ChϕA—as compared with ordinary conditions The least susceptible to aeration is ChϕB

2 Influence of aeration on the resulting number of bacteriophage corpuscles The platings were made from the tubes used in previous experiment after 24 hours It was found that increased aeration also greatly increased the resulting number of corpuscles—usually about 10 times, sometimes reaching, as in case of ChϕA, even 20 times

3 Influence of aeration on the virulence of bacteriophage To determine this, different types of Ch ϕ were daily transferred on the one hand in Legroux tubes and on the other in 7 inches × 0 75 inch tubes where the height of the liquid reached 7 5 cm After 5 and after 10 such transfers the virulence of the two obtained races was compared by means of the virulence test performed for both in our ordinary 8 inches × 1 inch tubes No increase of virulence was obtained with our standard Ch ϕ ϕ As, however, it is probable that our standard races already reached the maximum of virulence, these experiments cannot be considered to be conclusive

The range of action of bacteriophage

It is not often that we can find a bacteriophage of good virulence which will at the same time attack all the strains of a bacterial species Such cases are common only amongst dysenteryphages and that only in respect of the typical representatives of the species which are homogeneous Already with *V cholerœ* which is less homogeneous, even using 'smooth-rough' bacteriophage like Ch ϕ B, we meet vibrios which are not attacked by every race In the case of Ch ϕ A this restriction in the range of action is still more pronounced—Ch ϕ 61A cited further on provides a good example of this

However, in the case of Ch ϕ its adaptation to the naturally resistant strains, provided they are of the required phase of dissociation, and are not the result of acquired resistance to that particular type (i e , are not the secondary growths

after the action of a bacteriophage of the same type), presents no difficulty Our technique for adaptation in these cases is the following —

A relatively small quantity—0 5 c c to 1 c c —of a very young (4 to 6 hours) culture of vibrios to which our bacteriophage is to be adapted is added to 50 c c of broth in a 200 c c flask (thin layer of the medium for thorough aeration) and inoculated with a very large amount of bacteriophage suspension (10 c c to 20 c c) After 3 to 6 hours to this flask is added 2 to 5 c c of a young culture sensitive to our bacteriophage and all is left overnight Next day 20 c c from the flask is filtered and the procedure repeated At the same time direct platings (' big loop ' of the filtrate is spread and then covered with culture) of this filtrate, using the resistant vibrio are made, to see whether the adaptation has taken place If the clearings appear, they often present themselves as only ' traces '—small areas completely covered with bacterial growth In this technique it is advisable to use slightly moist agar for platings Though it makes the clearings spread and lose their shape, it provides us with a larger amount of bacteriophage material The clearings are scraped and washed into the as-yet-unfiltered culture of the previous day, which is then filtered to be used for the next transfer This considerably augments the number of adapted corpuscles in the filtrate From the moment the action of our bacteriophage on the resistant culture is established, the addition of sensitive culture is discontinued and the amount of filtrate for transfers gradually diminished (10 c c , 5 c c , 1 c c , 0 1 c c , ' bl ') The last two transfers can be carried out in test-tubes

If after 10 transfers the bacteriophage does not show adaptation, the transfers are discontinued and started anew with the same bacteriophage but propagated on another strain, which is used also for the second ' feed '

The history of adaptation of one of our races of Ch ϕA is interesting as illustrating how the range of action of a bacteriophage can be increased In 1929 we possessed a race of choleraphage (Ch ϕ 61A) recovered from a filtrate 11 months old (*see* under ' Stability ', (page 1186) It was the most virulent race we had at that time, but its action was restricted to only about 30 per cent of strains of *V cholerae* isolated in Patna and Calcutta A freshly isolated culture which was not lysed by this bacteriophage was taken and Ch ϕ 61A was adapted to act on it in the manner described above, and labelled Ch ϕ 62A After this adaptation to a single strain, it acquired the power to lyse all the cholera strains (about 200) we had at our disposal at that time the 30 per cent lysed by Ch ϕ 61A, and the remaining 70 per cent This suggested the existence of two different groups of *V cholerae*—one lysed by Chϕ61A and the other not lysed by this bacteriophage but lysed by Chϕ 62A During the whole year no vibrios were met with which were not attacked by Ch ϕ 62A Later on a parcel of 25 freshly isolated cultures of vibrio was received from Madras and not one of these was sensitive to Ch ϕ 62A The adaptation procedure was started using batches of 6 of these cultures The adaptation to the first 6 failed even after 10 transfers Another batch of 6 cultures was taken and after 6 transfers

we obtained the clearings with only one of them After a few transfers on this vibrio the newly adapted race was tried on all the 25 previously resistant strains all of them were lysed completely, *including the six of the first batch* and the five of the second, the adaptation to which had at first failed The adapted race of bacteriophage was labelled Ch ϕ 63A Its virulence against the other vibrios did not diminish Six months later 5 vibrios were isolated at Puri during the Jaggarnath festival, which were touched neither by Ch ϕ 61A, nor by Ch ϕ 62A nor by Ch ϕ 63 A The adaptation of the last to one of these Puri strains of vibrios was sufficient to make it active against all of them During later years Ch ϕ 64A was adapted to seven more varieties of vibrios without losing the virulence towards the rest of them and now is labelled Ch ϕ 71A After adequate passages through cholera patients this race of Ch ϕ A is used in our mixture for practical purposes

So much for the adaptation of bacteriophage to the different strains of the same species The question of the activity of a bacteriophage against the members of another genus is considerably more complicated On the one hand it is a well-established fact that one bacteriophage can act upon bacteria belonging to different genera a dysenteryphage often attacks *B coli* and salmonellas, and so on On the other, the question of the possibility of adaptation of bacteriophage to bacteria belonging to widely different genera is not yet settled, particularly after the recent publication of Beard (1931) d'Herelle, Malone, and Lahiri (1929) stated that usually Ch ϕ is also active against *B coli* if tried on the strains of the latter isolated from the same patient Our researches in this direction made on about 200 specimens of stools from cholera patients do not allow me to corroborate that statement I did not succeed in isolating one single race of Ch ϕ which was active also against *B coli* Our technique in these investigations was as follows —

The specimens of stools were collected from a cholera patient soon after his admission to the hospital and then every day as far as possible Vibrios and five colonies of *B coli* were isolated from the first stool When no more stools were obtainable from this patient, filtrates were prepared from all the specimens collected and were tested as to their activity for auto-strains' of *V choleræ* and *B coli* The filtrates which were active against both were each transferred separately on the same *V choleræ* and *B coli* In the majority of cases as soon as the third transfer and not later than after 5th, the activity of the filtrate restricted itself to the organism on which it was transferred one transferred on *V choleræ* lost its activity for *B coli*, one transferred on *B coli* lost its activity for the vibrio This means that the filtrates of dual action actually contained two different bacteriophages Ch ϕ and Coli ϕ

However, on no account can I consider these experiments as conclusive Very soon after the beginning of our activities in the hospital at Puri where we collected the largest proportion of our material, the hospital was 'contaminated' with

our own bacteriophages, which were freely distributed in the town amongst the pilgrims These bacteriophage races were not active against *B coli* but very virulent for *V cholerae*, and could easily have suppressed the less virulent races of bacteriophages naturally present That means that in the large proportion of cases we were examining again and again our own bacteriophages, multiplied in the intestines of the patients, missing the 'natural' ones I am quite prepared to see that experiments performed in 'fresh' localities, where new races of Ch ϕ can be found, will enable one to find a Ch ϕ active against *B coli* as well

We also failed in our efforts to adopt our Ch $\phi\phi$ as well as some freshly isolated ones to act on *B coli* However, I think it of interest to mention one of the methods of adaptation we used It may prove useful for other investigations —

The principle of the method was to influence the composition of a resistant bacterial body in such a way as, on the one hand to make it approach to the composition of the sensitive species, and on the other to influence the composition of the sensitive bacterial body on which bacteriophage is propagated, approaching it to that of the resistant, giving to the bacteriophage a chance of accustoming itself to the new substance

For this purpose two 'pure bacterial' media were prepared A large amount of *B coli* culture on the one hand and *V cholerae* on the other were obtained on the surface of agar, scraped (and not washed, to obviate the extraction of nutrient material from the agar) and emulsified in saline Phenol red was added as usual, reaction brought to pH 6 8 to 7 0, and the emulsion distributed in small tubes by 2 c c quantities and sterilized at 110°C for 15 minutes In this way each medium had as nutrient material only the bacterial substance

V cholerae were cultivated on 'coli-medium', and *B coli* on 'cholera-medium' In this way, making *B coli* assimilate cholera substance, and *V cholerae* the coli substance I tried to approach the composition of the vibrio and *B coli* one to another Vibrios and *B coli* grew quite well, very soon making the medium strongly alkaline The technique of adaptation described above was used, but with smaller quantities of media and filtrate 0 5 c c of *B coli* growth on 'cholera-medium' was added to 2 c c of 'cholera-medium' and inoculated with 0 05 c c to 0 5 c c of the previous filtrate After 4 hours, 0 5 c c of vibrio grown on 'coli-medium' was added

Two experiments of 20 transfers each failed to adapt two races of Ch ϕ A and four experiments also of 20 transfers failed to adapt a mixture of all five types of Ch ϕ to act on *B coli* (four varieties) These experiments also I cannot consider as conclusive

Of similar order to the adaptation of bacteriophage to different strains of bacteria of the same species, is the problem of increasing the range of activity of bacteriophage towards members of the same culture possessing different degrees

of sensitivity or of resistance The wider range of action of a bacteriophage results in more complete and more permanent lysis, as in this case bacteriophage destroys a larger proportion of a culture

I have already mentioned the method of Seitz and Gough (1930) who increased the range of activity by gradual adaptation to members of the same culture more and more resistant I have devised the following methods for the same purpose —

d'Herelle noted long ago that in some cases the secondary growth can be spontaneously lysed again and bacteriophage in that case becomes more virulent We have noticed this also but particularly in cases where the bacteriophage culture was filtered through a defective candle A growth appeared in the filtrate and was then sometimes again spontaneously lysed If filtered it yielded a bacteriophage of increased range of action Out of this observation two methods were devised —

(*a*) A very large amount of bacteriophage (2,500 c c) is prepared in a balloon and filtered through a candle known to be defective The test has to be left to a chance appearance of the growth in the filtrate and subsequent lysis The last did not happen often, but it is worth while to persist and repeat this experiment as, once it is successful, the increase in the range of action of bacteriophage is very pronounced

(*b*) A large amount of bacteriophage suspension (500 c c) is filtered into 2,000 c c of broth, using the same device as for examination of large quantities of water Before filtration some 10 c c to 20 c c of bacteriophage suspension with secondary growth is put aside and added to the flask after filtration A quick growth appears which is sometimes followed by lysis and we again can ascertain a considerable increase in the range of action If no lysis happens 500 c c from this flask is again filtered into 2,000 c c of broth to which this time is added a large amount of 'normal' sensitive bacterial culture—some 100 c c The experiment is continued in this intermittent manner After one filtration some 10 c c to 20 c c of saved secondary culture is added to the diluted filtrate, after the next 100 c c of sensitive culture, then secondary growth again, until a definite lysis of the 'secondary culture' is observed

Another method is based on the observation that sometimes bacteriophage acts better on the surface of the agar than in a liquid medium This can be seen when a young secondary growth is spread in a continuous layer on agar After incubation in many instances it can be seen that some of the corpuscles of bacteriophage are able to overcome the resistance of the secondary growth and produce 'secondary' clearings (Plate LVI, fig 3) If these clearings are very numerous, the whole growth is scraped and washed away with broth, filtered, and to the filtrate a small quantity of the secondary culture is added If the 'secondary clearings' are not numerous, they are picked up into broth in the same way as bacteriophage is isolated from platings To the broth, without preliminary filtration, a small

amount of secondary growth is again added The process is repeated until a *definite increase in the range of action is found*

Stability of bacteriophage

It is well known that some bacteriophages can be preserved in a filtrate for a very long time The best example of this was observed in Dr d'Herelle's laboratory* when an ampoule of d'Herelle's original Shigaϕ, preserved since 1917, was opened in 1930 and it was found almost as active as at the time of sealing But it is much less known that some bacteriophages are very unstable I have had quite virulent Ch$\phi\phi$ which died out completely within a few days Studying the stability of bacteriophage I came to the conclusion that, at least so far as it concerns Chϕ, a freshly isolated ChϕA is extremely unstable, and can die out in a few days' time I have fewer observations on ChϕB and ChϕC but in general the former of these seems to be the most stable of all However, stability is a property of individual corpuscles and varies considerably not only from race to race but from one corpuscle of the same race to another The most important observation is that while a freshly isolated race is rather unstable, the same becomes more and more stable the longer it is cultivated under laboratory conditions In this way an old laboratory race of bacteriophage will be very stable and will preserve its activity with little loss even for years

This constitutes a problem which is not yet completely solved As I have insisted on so many occasions for practical application we must use bacteriophages as little influenced by our artificial methods as possible If we use freshly isolated races they will die out before reaching the patient We tried to solve the problem in the following way We preserved our laboratory 'educated' races of bacteriophage for a very long period—many months During this period the less stable corpuscles were expected to die out, leaving the more robust ones These old bacteriophages were then used for human passages which we considered would render them more natural It is true that this stable bacteriophage after these passages suffers some loss of stability, but it still remains considerably more stable than a freshly isolated 'natural' bacteriophage

Estimation of activity of bacteriophage suspension

As I have already stated we cannot measure the virulence of a mixture of bacteriophages of different types by the rate of multiplication Even in the case of a pure-line bacteriophage suspension where the virulence (individual property of bacteriophage corpuscle) varies from one corpuscle to another, when we take the rate of multiplication as an indication of virulence we are to a certain extent

* Personal communication by Dr V Sertic

stretching the truth, considering the rate obtained as the average virulence of corpuscles of that race In the case of a mixture of types where the rate of multiplication varies so widely (from $n \times 10^6$ to $n \times 10^1$ in case of Chϕ) it is useless to work out an average as it will not convey any meaning and will not correspond to the actual action of the mixture

But if we cannot measure the actual virulence of a mixture, we can compare the activity of mixtures and can obtain an idea as to which mixture is more destructive At present, until the technique of estimation of virulence by the rate of destruction of bacteria is worked out, we can estimate the activity of a mixture only by the rapidity and permanency of lysis

We use the following technique for determination of the activity of our mixture of Ch$\phi\phi$ prepared for field use —

Standards of turbidity —The description of lysis by a certain number of crosses as in the Wassermann reaction is not sufficient as often we have to note very fine differences in the degree of lysis It is essential to have standards of turbidity with which we can compare our tubes The turbidity standards prepared with barium sulphate after Brown are not satisfactory for our purposes as barium suspensions are definitely grayish in comparison with the bluish growth of *V cholera* in peptone water We prepare our standards with the vibrios themselves The difficulty is in preventing the autolysis of the vibrio, which soon appears even in formalinized cultures We first prepare a very heavy milky emulsion of cholera vibrios by washing the agar growth with peptone water To this emulsion 1 per cent formalin is added and all is sealed in a large ampoule and heated at 80°C for half an hour This prevents to a considerable extent further changes after the emulsion is properly diluted The dilutions are then made using peptone water as diluent with the help of Brown's turbidity standards (Burroughs Wellcome & Co) to contain 1×10^9, 5×10^8, $2\,5 \times 10^8$, $1\,25 \times 10^8$ and $6\,25 \times 10^7$ vibrios per c c The first tube is labelled '0', the second '2', then '4', '6', and '8' A tube with pure peptone water is labelled '10' Ten c c of each dilution is put in test-tubes of the same size as those in which the activity test is performed and the tubes are sealed in the flame

Activity test —The test is performed in 7 inches $\times$ 0 75 inch tubes containing 9 c c of 1 per cent peptone water One c c of 16 to 18 hours old culture of vibrio in peptone water is added and is inoculated with 1 drop of bacteriophage mixture At least 4 cultures are used for each investigation—2 freshly isolated smooth and two rough Our rough cultures are so chosen that one is particularly sensitive to ChϕB the other to ChϕC The observations of the lysis give a fairly good idea as to the activity of the mixture

It is to be noted that in this case we compare equal quantities of bacteriophage suspensions giving us an idea of the activity of the mixture and not of the virulence of the bacteriophage

SUMMARY

The virulence of bacteriophage is considered as an intrinsic property of the bacteriophage corpuscle, and following d'Herelle's definition is accepted as its power to develop within and at the expense of bacteria, causing their destruction The degree of virulence is considered the higher as multiplication and destruction is more rapid

The fallacies of the different methods for determination of the virulence of bacteriophage are discussed and a new virulence test, based on determination of the rate of multiplication, is suggested It is shown that the new virulence test gives also other possibilities for the study of bacteriophage properties The technique of its varied practical applications is described

The influence of environment on the propagation of bacteriophage and on its virulence are described and methods for its increase are suggested

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BIOLOGICAL ASSAY OF DIGITALIS PREPARATIONS IN THE TROPICS

Part III

BY

LIEUT-COLONEL R N CHOPRA, M A, M D (Cantab), I M S,

AND

CAPTAIN J S CHOWHAN, M B, B S, A I R O

(*From the Department of Pharmacology, School of Tropical Medicine and Hygiene, Calcutta*)

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CHOPRA AND DE (1926, 1926*a*) pointed out that it was not always possible to obtain the suitable species of frogs—*Rana temporaria*, *Rana pipens* or even *R esculenta*—for assay of digitalis preparations in India Besides this the seasonal variations in the sensitiveness of frogs are much more marked in tropical climates and these amphibia give widely divergent results These workers, therefore, found the 'frog method' of assay unsatisfactory and discarded it entirely in favour of Hatcher and Brody's 'cat method' With the latter method also they were much disconcerted by the fact that some preparations which gave very high values on assay were found to be useless on therapeutic trial Chopra *et al* (1925) showed that the physiological activity of tinctures of digitalis deteriorates rapidly in warm climate such as that occurring in India When tested by the 'cat method', in which the diluted tincture is given intravenously, such tinctures may kill the animal with a small minimum lethal dose and thus show high activity, yet when tested by the guinea-pig or the frog method, in which the drug is given

subcutaneously, they show a marked reduction in their potency Some chemical changes, so far undetermined, take place in these tinctures which decrease the quantity of therapeutically active glucosides, leaving the toxic elements unaffected It should be understood that Hatcher and Brody's (1910) method and some of its modifications, give an indication of the amount of active principles of digitalis that are lethal to the animal It is by no means certain in the tropics that the active principles which produce death are the glucosides which cause therapeutic effects, though in freshly prepared tinctures there is a distinct relationship between the two Samples of tincture, which in small doses are quite strong enough to be lethal to cats when given intravenously, are useless when administered by the mouth to man

For these reasons the senior author (R N C) introduced a modification of Hatcher and Brody's 'cat method', in which besides the lethal dose, some other indications of physiological activity can be gathered A short description of this method for ready reference will not be out of place here

Method

The animals selected are healthy cats weighing between 1,600 to 2,500 grammes These are starved for 12 hours before use and are anæsthetized by intraperitoneal injection of 0 18 to 0 2 g of chloretone per kilo body-weight dissolved in a few c c of alcohol, the animal goes under deep anæsthesia in 20 to 30 minutes Anæsthesia can also be induced by giving chloralose by a stomach tube, the animal getting under deep anæsthesia in 1½ to 2 hours The tinctures are diluted with nine parts of physiological saline solution and physical characters of the solution are carefully noted The assay is carried out in the usual manner, the injection being given in the femoral vein at a regular and uniform rate of 1 0 c c of the diluted tincture per kilo body-weight in 3 to 5 minutes The solutions should always be filtered through a pledget of cotton-wool placed at the botton of burette before injection into the femoral vein The animal is closely watched, the heart is carefully auscultated with a stethoscope every few minutes and any changes in the character and frequency of the rhythm are noted and the beats per minute recorded From an analysis of the data thus collected, it is possible to form an idea of the physiological activity of the preparation apart from its toxic effects In carrying out assays by this method, special attention should be paid to the following opints —

1 The amount of tincture required per kilo body-weight to produce a well-marked slowing of the heart

2 The degree of slowing

3 The total duration of slowing or digitalis action

4 The time required for the completion of assay and the total amount of tincture per kilo body-weight necessary to produce lethal effects

It should be noted that no single factor mentioned above gives a true conception of the potency of the tincture All four points should be taken into consideration in conjunction with one another

The average rate of heart-beat in cats anesthetized with chloralose varies from 150 to 180 per minute The first effect of injection is an increase of 10 to 15 beats within a few minutes, but then the beats begin to slow down and the minimum is reached in 20 to 30 minutes The sounds during this stage are clear, loud and forceful The duration of slowing, which is an indication of physiological action of digitalis, is 15 to 30 minutes after which the rate is again quickened The sounds henceforward become weak at first and later muffled and indistinct, the beats become irregular, feeble and at this stage the administration of digitalis should be stopped

With good physiologically active tinctures the frequency of the heart-beat falls perceptibly with about 2 5 to 5 c c of the diluted tincture per kilo body-weight and the degree of reduction is considerable The reduction may be as much as 50 beats or more per minute but at an average it is not less than 20 beats per minute with active tinctures The time required for completion of the assay is about 1 to 1½ hours In case of weak tinctures, as a rule, more of the diluted tincture is required per kilo body-weight to produce slowing, or digitalis action, and the degree of slowing and its duration are less as compared with tinctures of standard strength The time taken to complete the assay and the amount of tincture used are also larger

As regards the physiological activity of tinctures which have become toxic on account of climatic conditions, it is very difficult to form any accurate idea That a tincture has become toxic is shown by the fact that the whole course of assay is altered The slowing, if it is produced at all, sets in rapidly, its duration is short, the assay is completed and the animal dies in a much shorter time, i e , considerably less than an hour Here the physiological effects produced by the glucosides on the heart muscle become overshadowed by the toxic effects

It may be observed that though cats are readily procurable in Calcutta, a very careful selection should be made before they are actually utilized for standardization experiments Many of the cats brought to the laboratory are diseased and are unfit for assay work Besides, many animals show abnormal individual variations Thus the rate of heart-beat, the slowing produced and the lethal doses vary with the sex, colour and season Tiger cats and those which are jet black in colour are resistant to digitalis group of drugs both as regards the slowing produced and the lethal dose Female cats are easily baited, being more domesticated but when pregnant and lactating they are abnormally resistant and unsuited for this work The animals weighing less than 1 5 kilos and above 3 kilos are also not suitable and should be discarded All animals used for assay purposes should be kept under observation for 2 or 3 days and should be starved for 12 hours before assay

Frequently it is possible to complete the assay of a preparation with one animal only With a little practice it is quite easy to tell from the variations in the character of the sounds, slowing of the rhythm, duration of slowing, etc , whether the preparation is physiologically active or otherwise If, however, unsatisfactory results are obtained, a second animal must be used to confirm these results If the results obtained from the first cat can be confirmed by the second, there is no necessity for further trials, but if they are discordant, a third animal must always be used to give a final verdict

Good samples of tinctures of digitalis have a sweet and slightly aromatic odour when diluted 9 times with normal saline , they are slightly opalescent and have a bright greenish yellow colour In the majority of cases the physical appearance of the diluted tincture, as has already been stressed before, gives valuable information as to whether it is physiologically active or has deteriorated Those having a deep green, blackish brown or muddy colour with floating particles are usually *below par* and not unfrequently toxic Such changes, though not common in tinctures stored in temperate climates, frequently occur under tropical conditions

The results obtained by this method have been carefully checked by therapeutic trials of tested tinctures on patients suffering from cardiac disease, the amount of tincture required to get a patient under digitalis effect being the criterion of its potency This clinical test gives the most reliable information regarding the physiological potency of a tincture The average dose of a good active B P tincture required to get a patient under digitalis effects is 15 c c (4 drachms) per 100 pounds (50 kilos) of body-weight in 36 to 48 hours With weak tinctures both the dose and time are considerably increased

Our object in this paper is to show that the results obtained with this method run practically parallel with the standard methods of assay prescribed by the League of Nations Standard digitalis powder prepared by Professor Magnus according to the directions of the First International Conference on Biological Standardization (1923) was obtained from the National Research Council, Hampstead, London The M L D of this powder for cats is 89 7 mg per kilo body-weight, i e , 1 0 g of the powder represents 11 1 fatal cat doses This powder is intended to be the basis for working out the standards in different countries and the recommendation is that ' only such leaves shall be passed for issue which differ from the standard preparation by not more than 25 per cent ' From this powder an infusion was prepared according to the method of the Dutch Pharmacopœia, 1 0 g of the powder being dissolved in 200 c c of distilled water and raised to 90°C for 15 minutes and then filtered This solution represented 5 mg of the standard digitalis per c c This was made iso-tonic by adding sodium chloride, cooled and kept in the dark at a temperature of about 10°C to 15°C The solution was freshly prepared every third or fourth day to avoid chances of deterioration With this standard solution

we carefully assayed ten cats taken at random without selection The following table gives a summary of the results obtained —

TABLE I

Sex, character and weight of cat		Death Interval		Slowing of heart beats per minute	M L D of digitalis in mg per kilo body weight 5 mg = 1 c c of infusion
		Hours	Minutes		
1 Female	1 740 g	1	10	40	57 5
2 Male, white and brown	1,720 „	1	12	20	68 0
3 Male, white	1,880 „	0	55	16	63 5
4 Female, white and black spots	1,990 „	1	20	60	65 35
5 Male, white	1,675 „	1	10	28	62 5
6 Male, white and black	1,425 „	1	5	10	68 20
7 Female, brown and white	2 510 „	1	25	68	74 5
8 Female, tiger spots	2,200 „	1	45	20	74 0
9 Female, white	1,625 „	1	30	20	80 0
10 Female, white and black	1 830 „	0	50	20	50 0
AVERAGES	1 868 5	1	18	30 2	66 35

A perusal of Table I will show that the reduction was poor in numbers 3 and 6 This was probably due to variations in the sensitiveness of the animal as the cats 4, 5, 7 and 8, done with the same solutions, gave satisfactory reduction According to Burn (1928) the lethal dose must be calculated as the average of several experiments and slowing of heart-beat in our experiments was calculated in a similar way The average must approximate more closely to the true value as the number of cats is increased The average death interval in this series was 1 hour 18 minutes, the average slowing of the heart-beats 30 2 per minute and the average lethal dose 66 35 mg of the digitalis leaf per kilo body-weight of cat The M L D for Indian cats from this series would appear to be considerably smaller than the average for European cats, i e , 66 35 as compared with 89 7 mg per kilo body-weight

In Table II we have given the results of assay of 163 tinctures of digitalis carried out by this method in our own laboratory It will be observed that 123 tinctures were passed as being up to the B P standard and in these the average reduction in the number of beats amounted to 42 5 per minute (as compared with 30 2 of the standard international digitalis leaves in Table I) The average death interval

was 1 hour 11 minutes as compared with 1 hour 18 minutes and the average lethal dose was 60 04 mg per kilo body-weight as compared with 66 35 mg of the standard international powder (European standard 89 7 mg) The low figures in both cases might be due to the development of toxic changes

TABLE II

Preparation tested	Total number of samples assayed	Average weight of cats, g	Average lethal dose per kilo of body-weight, c c	Slowing of heart-beats per minute	Death interval		M L D of digitalis in mg per kilo body weight 5 mg = 1 c c of infusion
					Hour	Minutes	
National standard	10	1,868 5	13 2	30 2	1	18	66 35
Up to the B P standard	123	2,014	11 36	42 5	1	11	60 04
Below B P standard	40	1,918 7	11 92	15 9	1	1	60 52

Of the 40 tinctures which were not up to the B P standard, the average slowing of the heart-rate was 15 0 beats per minute and the death interval was 1 hour 1 minute These tinctures were declared below standard, although the lethal dose was 60 52 mg per kilo body-weight, which according to the ordinary cat method would be up to standard Our conclusions, however, were supported by the fact that in this group the whole course of assay was abnormal and showed that there was something wrong with the preparations under test Many of these tinctures showed physical changes in their appearance and the lethal interval of only 30 to 45 minutes indicated toxic changes Eighteen out of forty showed little or no slowing of the heart-beat , these tinctures were evidently weak as the death interval was long and the lethal dose was high

SUMMARY

It will be seen from what has been said, that it would be very difficult to carry out biological assay of digitalis preparations in the tropics with any degree of accuracy, if one adhered to the methods and standards laid down in temperate climates Of the two methods recommended by the Health Organization of the League of Nations the frog method has been found to be unworkable in practice, at least in Calcutta The cat method as ordinarily used has also its limitations as it gives no idea regarding the tinctures which have deteriorated and become toxic Chopra's modification of the cat method described in this paper, gives a satisfactory indication of the physiological activity of the preparation The results

obtained in a series of 163 tinctures ran practically parallel with the other standard methods with the additional advantage that a fair indication of the physiological activity could be gathered apart from the lethal dose

It is also necessary to formulate fresh standards in India For instance the minimum lethal dose of the standard digitalis powder for European cats per kilo body-weight is 89 7 mg , while in a series of 163 preparations examined in Calcutta it worked out as 60 26 mg per kilo body-weight with the Indian digitalis leaf With the standard digitalis leaf it was 66 35 mg per kilo body-weight in 10 animals, that is 1 0 g of the leaf represented 14 9 fatal cat doses instead of 11 1 The cats obtained in Calcutta appear to be more susceptible to the toxic effects of digitalis and it is likely that there may be similar variations in different parts of India The pharmacological laboratories carrying out assay work should therefore work out their own standard before undertaking this class of work

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COMPLEMENT-FIXATION IN FILARIASIS

BY

LIEUT.-COLONEL R B LLOYD, M A, M B, B Chir, I M S,
Imperial Serologist, Professor of Serology and Immunology,

AND

S N CHANDRA, M B,
Assistant Serologist, Assistant Professor of Serology and Immunology,
School of Tropical Medicine and Hygiene, Calcutta

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INTRODUCTORY

THE present investigation was undertaken to examine how far the complement-fixation test is of value in the diagnosis of infections with *Wuchereria bancrofti*. While we have found that the test is of distinct value for this purpose, it is probable that the main interest of our results will lie in their application to the general pathology of filariasis.

The literature of the subject is very limited. The earliest studies on the immunology of filariasis were those of Taliaferro and Hoffman (1930) who worked out a dermal test using as antigen extracts of *Dirofilaria immitis* obtained from the dog. The outstanding feature of Taliaferro and Hoffman's work was that the occurrence of a dermal reaction was demonstrated in persons with microfilariæ in the blood as distinct from cases of clinical filariasis. The first work on complement-fixation in filariasis was that of Fairley (1931) who also used an antigen prepared from *D. immitis*. In a series of seventy cases Fairley obtained eleven positive reactions. Six of these were in cases of *Loa loa* from the West Coast of Africa. Four were cases of active or latent infections with *W. bancrofti*. Of these four, only one was suffering from a filarial condition alone, the remaining three suffering

also from syphilis, kala-azar and carcinoma respectively We do not see particulars of the remaining positive case Of fifty-nine cases which yielded a negative reaction only three appear to have been cases of *W bancrofti* infection, all being old cases Excepting one case of *Onchocerca volvulus*, the remaining fifty-five cases were non-filarial conditions which included a number of other helminthic diseases The inclusion of these latter is useful as a control The series of *W bancrofti* infections examined appears therefore to have been one of eight cases, of which five reacted positively and three negatively In a discussion on a paper by O'Connor (1932), Fairley is reported as having stated that 'Regarding the complement-fixation reaction with *Dirofilaria immitis* antigen, though this is almost always strongly positive in *Loa loa* infestation, it has yielded disappointing results in a considerable number of sera collected in India from patients showing microfilariæ, only five out of twenty-three cases yielding positive results Dirofilaria extract therefore cannot be regarded as a satisfactory antigen for detecting circulatory anti-body in filariasis'

Methods.

For the complement-fixation tests in filariasis we have employed the Wassermann technique which has been in use in the Imperial Serological Department, Calcutta, for many years, modified as to dosage of reagents as was found necessary for this particular purpose As antigen we first used alcoholic extracts of powdered dry *D immitis* Later we attempted unsuccessfully to enhance the sensitiveness of this antigen by the addition of cholesterol Subsequently, we carried out the tests with two antigens side by side, viz, (1) the plain alcoholic extract of *D immitis* as above, and (2) an antigen derived from the acetone-insoluble lipoids of *D immitis* obtained by exactly the same process as that used by Boidet and Ruelens in the preparation of their well-known antigen for the diagnosis of syphilis by the Wassermann reaction Experience with complement-fixation in tuberculosis and other diseases has indicated that the more specific lipoids are to be found in the acetone-insoluble fraction and we have been very satisfied with the results obtained with this latter antigen, which is undoubtedly to be preferred to the plain alcoholic extract We may remark that *D immitis*, though not the cause of human filariasis, is the only worm of the filarial family which is obtainable in quantity, and therefore we have no choice but to use it In the circumstances the test could not be expected to be more than a group reaction for filarial infections generally We shall return to this point later

Routine examination of all filarial cases, both clinical and of the stool, is necessary to estimate how far an eosinophilia may be due to the presence of other helminths The percentages given in this paper for the differential white counts were based on counts of a minimum of three hundred leucocytes

SERIES EXAMINED

The series of cases examined was at first entirely unselected, except that as far as possible we excluded cases in which other diseases were also present Later, when it became evident that old cases of elephantiasis almost always yield a negative reaction, we ceased to examine these and concentrated on the examination of the more acute types The complement-fixation tests carried out by us were one hundred and seventy in number performed on a series of one hundred and four cases as detailed in the subjoined table Of these, eighty-nine were conditions due to *W bancrofti* infection, the remaining fifteen cases being infections by other helminths examined as controls The classification of the filarial cases examined into clinical types is necessarily very rough, since there is much overlapping, and even in the same case one type of lesion may predominate at one time and another at another The difficulty is greatest in deciding whether to regard a case as one of chronic elephantiasis or of acute lymphangitis, since acute attacks are constantly superimposed on the chronic conditions The criterion we applied was to regard as acute cases those in which we were able to examine the blood during an actual attack Where more than one type of lesion was present we classified the case according to the most prominent lesion While the clinical differences shown in Table I must be regarded as broad distinctions only, it is, we think, useful as indicating the clinical types in which the positive reactions are chiefly found

It will be seen that old cases of elephantiasis without evidence of recent attack practically always yield a negative complement-fixation reaction The same is true of a type of slowly developing lymphangitis of leg which is common in young women in Calcutta These are not old cases, but a swollen leg gradually develops without any acute attack The blood count in both these types shows neither eosinophilia nor leucocytosis, being ordinarily normal As regards the more acute types, acute cases of filarial lymphangitis which seemed similar clinically reacted, some positively and some negatively, to the complement-fixation test in a manner which was at first sight very puzzling In contrast to some acute cases which yielded a negative reaction we have found several in which the attack was distinctly sub-acute, but which yielded a strongly positive reaction The reason for the varying result of the complement-fixation reaction in this type of case was made clear when the blood counts were examined While there are some cases, even acute cases, of lymphangitis which show no blood changes at all, and others in which the only finding is the eosinophilia characteristic of the preceding symptomless phase of microfilariæ in the blood, both these types being associated with a negative complement-fixation reaction, the main bulk of the acute and sub-acute cases fall into one or other of two types which are associated with quite distinct blood pictures In the first, which we have termed the toxic type of attack, the finding is a positive complement-fixation reaction associated with an eosinophilia, the total white

TABLE I

Analysis of results

Result of the complement-fixation test in various types of filariasis and other helminthic infections

	Positive	Negative	TOTAL
A Conditions due to *W. bancrofti* infection			
Elephantiasis	2	24	26
Chyluria	3	2	5
Intermittent hæmaturia	0	1	1
Superficial lymph varices	0	5	5
Acute and sub acute attacks of lymphangitis	10	17	27
Filarial fever	1	1	2
Slowly developing cases of lymphangitis leg, not old cases but no attack	0	4	4
Hydrocele and cyst cases	4	6	10
Lymphadenitis	2	0	2
Unclassified	1	0	1
Filarial infestation	0	6	6
TOTALS	**23**	**66**	**89**
B Conditions not due to *W. bancrofti*			
Guinea worm	3	1	4
Hookworm	0	10	10
Tape worm (*Tænia saginata*)	0	1	1
TOTALS	3	12	15
GRAND TOTALS	**26**	**78**	**104**

count and the percentage of polymorphonuclear leucocytes being within normal limits In the second, which we have termed the septic type of attack, we find a negative complement-fixation reaction with little or no eosinophilia, and an increase both in the total white count and in the polymorphonuclear percentage The differences between the two types of blood picture are on the whole surprisingly clear cut, though a minority of cases show a mixed type of response, e g , a positive complement-fixation reaction associated with a polymorphonuclear leucocytosis One of our cases was examined during two acute attacks During the first attack a mixed type of response was met with During the second the response was of the purely septic type Attacks of the toxic type are not limited to the early stages of clinical filariasis, since we have found the positive complement-fixation reaction in cases with as much as four years' history of repeated attacks We may remark in passing that in the cases examined by us the leucocytosis was never very marked Even in those exhibiting a purely septic type of attack the total white count seldom exceeded eleven to twelve thousand per c mm Clinical appearances do not indicate with any certainty which type of attack is occurring, though the septic types tend perhaps to be the more acute The toxic type of blood picture while occurring in acute conditions of superficial lymphangitis indistinguishable from those caused by septic attacks, is found also in sub-acute inflammations such as some cases of hydrocele and in chyluria These clinical types do not ordinarily exhibit any acute symptoms to which the term ' attack ' could be applied

We have performed repeated complement-fixation tests on those cases showing a positive reaction in order to see how the latter behaves in relation to changes in the clinical condition In all such cases which show a clinical ' attack ' of lymphangitis the positive reaction regresses to negative as the attack passes off The basis of this statement is the result of complement-fixation tests performed on the ten positive cases shown in the table under the heading of acute and sub-acute lymphangitis

The five cases of chyluria examined all showed very definite eosinophilia, though the complement-fixation reaction was only positive in three The condition of chyluria commonly remains unchanged for long periods, but some cases do show slight clinical changes, at any rate in the amount of chyle discharged in the urine In one of our cases we happened to hit off a period during which the reaction while strongly positive originally weakened almost to a negative, the clinical condition having meanwhile practically cleared up In another of our cases the chyluria is becoming steadily worse, and the complement-fixation reaction remains strongly positive Whether the complement-fixation reaction is positive or negative, the eosinophilia is always very definite in chyluria Some cases of hydrocele also yield strongly positive reactions In this type of hydrocele the eosinophilia is invariably marked One very interesting case in which we were able to study the effect

of emptying the hydrocele upon the blood findings has provided important indications, and we reproduce the observations *in extenso* in Table II —

TABLE II

Date	Complement fixation reaction	Total whites	Eosinophils	Polymorph percentage	Presence of microfilariæ	Other helminths	Clinical.
27-10-32	+++ (night)						
28-10-32	+ ++ (day)	9,500	6	70	+ in blood + in hydrocele	Hookworm ova	Double hydrocele No acute symptoms
14-11-32		9,400	8	73			
17-11-32		10,200	14	65			
19-11-32		8,100	10	70			
21-11-32	+++						
22-11-32		7,900	11	68			Hydrocele tapped (completely emptied)
23-11-32		10,300	12	65			
26-11-32		9,100	11	65			
13-12-32							

The position here is that a case of hydrocele which has shown no clinical change yielded three positive complement-fixation reactions before the hydrocele was emptied Twenty-two days after emptying, the reaction was negative It is evident that the antigenic factor for the complement-fixation reaction has been removed by the tapping It will be interesting to see if the positive reaction returns when the hydrocele refills It will be noticed that the eosinophilia is unaffected by the tapping, but it is uncertain how far, in the present case, this is dependent on the filarial condition, since hookworm infection was also present, and the attempts made to eradicate the hookworm infection were not particularly successful

Discussion

The fact that in those cases of acute and sub-acute lymphangitis with a positive complement-fixation reaction the reaction becomes negative as the attack passes off, combined with the circumstance that the reaction of persons suffering either merely from microfilariæ in the blood, so far as examined, or from elephantiasis—the two extremes of the disease—is negative, suggests that the positive complement-fixation reaction is an immunity response characteristic of the attack, i e, that this type of attack is dependent on releases of filarial toxin It would also seem probable that this type of attack is precipitated by releases of filarial toxin which are, presumably, too great to be dealt with by the eosinophilia which is the typical response in the preliminary phase of symptomless invasion of the peripheral blood In short, the results of the complement-fixation test, considered with the blood counts, indicate that there are two causes of attacks of filarial lymphangitis (*i*) filarial toxin, and (*ii*) superadded sepsis In the latter, increased releases of toxin probably play little part As regards the latter type we have not touched the bacteriological side, but we assume septic infection to be present from the nature of the leucocytic response We see that both these causes may at times operate together to produce a mixed type of response

If we consider along with our own results the observation of Acton and Sundar Rao (1929) that eosinophilia is more characteristic of the preliminary stage of symptomless invasion of the peripheral blood than of the clinical filariasis which follows later, and the work of Taliaferro and Hoffman which showed that the dermal reaction against *D immitis* antigen may be obtained before symptoms occur, and the fact that this latter reaction is almost certainly of an anaphylactic nature, we can obtain some insight into the immunological responses occurring in man against the *W bancrofti* infection As stated, eosinophilia is particularly associated with the early stage of symptomless invasion of the peripheral blood It is also met with in anaphylactic shock The observed phenomena accordingly suggest to us that the sequence of events is probably somewhat as follows —

The immunological responses to the toxin in the early stage are, firstly, anaphylaxis and, secondly, an increase in the eosinophil output which is not limited to the peripheral blood since vast quantities of eosinophil cells may congregate round the adult worms The anaphylactic reaction, which is the most sensitive of all the humoral immunity reactions, appears first, being induced, we assume, by gradual small releases of filarial toxin whose products are probably disposed of in some way by the eosinophil cells During this early stage the less sensitive complement-fixation reaction does not detect toxin releases It is the usual immunological experience that anaphylaxis is the characteristic response when the dose of antigen is small, complement-fixation and specific precipitation being the responses when the doses of antigen are larger The development of a positive complement-fixation reaction during an attack of lymphangitis and its disappearance when the

attack is over probably imply that larger releases of toxin have occurred which have temporarily evoked a further stage of immunization We should accordingly expect to find that the eosinophilia, so characteristic of the preliminary phase, would continue during the further stage of immunization represented by the positive complement-fixation reaction With a single exception we have invariably found this to be the case The association with microfilariæ in the peripheral blood is naturally less close, since circumstances may exist which permit fluid leakage without escape of microfilariæ Probably the type of attack which supervenes and the presence or absence of eosinophilia are largely dependent on the degree of closure of the lymph channels and the amount of damage caused thereby As stated, three cases of chyluria out of five examined have shown a positive complement-fixation reaction and all showed marked eosinophilia In chyluria the closure of the lymph channels is never more than partial, and circumstances are presumably favourable to large releases of toxin continuous or intermittent

In view of the evidence we have adduced that some cases of acute and subacute lymphangitis are due to increased releases of the filarial toxin, we have to consider what possible explanation there may be of the fact that in fairly quiescent conditions due to *W bancrofti*, such as chyluria and some cases of hydrocele in which 'attacks' do not ordinarily occur and in which clinical changes are inconspicuous, we frequently find the blood changes (eosinophilia plus the positive complement-fixation reaction) which in other types of clinical filariasis due to this parasite are only met with during an attack This raises the question whether the anti-body demonstrated by complement-fixation is of a protective nature or not This is a difficult point, but the disappearance of the anti-body when the attack is over does not exclude its being so, since in syphilis the anti-body disappears, though not usually in this instance without treatment, and it is now believed that the Wassermann reaction is a specific anti-body reaction, the anti-body resulting from auto-immunization against the products of breakdown either of the tissues or the parasite or of both

We have spoken of the antigenic factor in filariasis as 'toxin', but we do not know that the poisonous excretion from the worm has any close relation to those products of bacterial activity to which the term 'toxin' is ordinarily applied The absence of eosinophilia during the early stages of bacterial invasions possibly implies that the two types of poison are chemically different The connection between the various types of anti-body is at present obscure, but we may remark that the diseases in which an effective anti-toxin is available are not those in which complement-fixation is most prominent

If the view we have put forward above as to the stages of immunization in *W bancrofti* infections is correct, it would seem likely that in the type of case represented by chyluria, where the lymph channels are still freely open, large releases of toxin may occur without producing any clinical attack, the body having acquired

a tolerance to these, and that attacks of peripheral lymphangitis in which the complement-fixation reaction is positive are caused by a sudden increase in the toxin released in areas where toxin releases are normally small, i e , in areas where the lymphatic blockage is extensive The distinction is of course not a sharp one, as serious exacerbations are not unknown in cases of chyluria We have also seen that cases of chyluria sometimes react negatively to the complement-fixation test This may possibly mean that the discharges of toxin are intermittent in such cases

The frequent occurrence of a positive complement-fixation reaction in chyluria may probably be associated with Fairley's finding that in a series of six cases of *L loa* infection every one gave a positive complement-fixation reaction with *D immitis* antigen The regularity of the positive reaction in *L loa* is presumably due to the fact that this parasite lives in the subcutaneous tissues and produces no lymphatic obstruction Our finding that of four cases of guinea-worm infection three gave strongly positive reactions, which were still positive nine months later, is probably to be interpreted in the same way All four cases of guinea-worm disease showed very definite eosinophilia This is exactly the same as the finding in chyluria, i e , the eosinophilia is always present whether the complement-fixation reaction is positive or not

From the nature of the antigen used it is not to be expected that the complement-fixation test will be able to distinguish between infections by *W bancrofti* and the allied *Dracunculus medinensis* On the other hand, it seems likely that the test will prove valuable in guinea-worm disease It may even prove more valuable in guinea-worm disease than in filariasis due to *W bancrofti* Cases of guinea-worm disease are very hard to come by in Calcutta, and this test should be taken up by those working in endemic areas, not only as a means of diagnosis, but also because it seems likely that a careful study of the immunology of guinea-worm disease may yield results which will have important applications to the more complicated case of infection with *W bancrofti* It is interesting that while the lipoids of the *Dirofilaria* antigen are highly specific, they yet by their group reaction afford serological evidence in support of the relationship between the two parasites (*W bancrofti* and *D medinensis*),—a relationship which is based on morphological characters

One very interesting case showed the toxic type of response in a patient with a five years' history of lymphangitis of scrotum with abscess with peric[illegible] Two cysts were also present, one in each axilla These were excised and contain adult worms After the excision microfilariæ appeared in the blood for the first time, the previously positive complement-fixation reaction became negative and the eosinophilia of 10 dropped to 4, even though hookworm ova were present This result strongly suggests that the cysts were the antigenic factor, since on their removal the complement-fixation reaction became negative It is not likely that the filarial abscess was the antigenic factor, since

were dead This case, without the complement-fixation findings, has been published by Sundar Rao (1933)

It will be recalled that in the rather similar case referred to earlier in this paper, in which emptying of a hydrocele changed the complement-fixation reaction from positive to negative, there was no change in the eosinophilia In both the cases hookworm infection was also present, and we cannot evaluate the effect of surgical treatment of the antigenic factor upon the eosinophilia due to filaria until cases are available which are free from infections with other helminths

The clinical phenomena produced by infection with *W bancrofti* are peculiar in that they largely consist of the results of lymphatic obstruction which ultimately leads to the partial or total exclusion of the antigenic factor from the general circulation In such circumstances we must not expect that a serological test could be devised which could diagnose all stages of the disease In many of the old cases of elephantiasis it is probable that the worms eventually become completely walled off from the circulation, thus effectually preventing any antigenic action As we should expect, eosinophilia is entirely absent in such cases

Diagnostic use of the complement-fixation test

It seems that the test will be of value in the examination of filarial conditions, more particularly in the detection of those cases of lymphangitis in which vaccines are not likely to be successful It may also be useful in bringing to light cases of latent filariasis A positive complement-fixation reaction is more valuable than the associated eosinophilia, since the latter may be due to other helminths, while the former is obtained only in filarial infections The test will consequently reach its highest usefulness in countries where helminthic infections are very common Since *D immitis* is not the cause of human filariasis, the test cannot be expected to be more than a group reaction for filarial infections generally, but, as the number of species in any one country is limited, this is not an important consideration

So far as we have seen at present, the complement-fixation test seems specific enough for ordinary purposes In every one of our tests Wassermann-positive sera were put up against the filarial antigen and in every instance reacted negatively The important question of cross-fixation between the Dirofilaria antigen and the serum of persons suffering from helminthic diseases other than filariasis has not yet been examined in detail Of twelve cases of hookworm infection ten reacted negatively The remaining two reacted positively One of these had in addition a filarial abscess with microfilariæ in the blood, being an obvious case of mixed infection The other case had not been recognized to be one of filariasis In view of the repeatedly positive serological finding, we asked for a complete clinical examination for signs of filariasis, and it was reported that the patient had enlarged epitrochlear and inguinal glands and that he came from Contai, Midnapur, which is a highly endemic area There is no doubt that this was a latent case of

filariasis which was brought to light by the complement-fixation test These two cases of mixed infection are shown in the table under the conditions due to *W bancrofti* One case of tape-worm infection (*Tænia saginata*) reacted negatively As stated, of four cases of guinea-worm disease three reacted positively

Summary of results and conclusions

1 A series of eighty-nine cases of various clinical types of infection with *W bancrofti* has been examined by the complement-fixation reaction, using as antigen extracts, variously prepared, of *D immitis* Twenty-three positive reactions were obtained A series of fifteen cases of infection by other helminths was also similarly examined, yielding three positive reactions, all of which were in guinea-worm disease

2 It is shown that cases of acute or sub-acute lymphangitis tend to exhibit two distinct types of immunity response In one type the finding is a positive complement-fixation reaction associated with an eosinophilia, the total white count and polymorphonuclear percentage being within normal limits In the other type the finding is a negative complement-fixation reaction with a polymorphonuclear leucocytosis We conclude, accordingly, that there are two distinct types of attack in such cases which we designate the toxic and septic types respectively

3 Repeated examination by the complement-fixation test of cases of acute or sub-acute lymphangitis with a positive complement-fixation reaction has demonstrated that as the attack passes off the reaction becomes negative

4 The positive complement-fixation reaction, in whatever type of filarial lesion it may be found, is always associated with an eosinophilia, and, so far as we can see at present, the disappearance of the positive complement-fixation reaction does not seem to affect the eosinophilia, though this point requires further examination

5 As a result of various considerations, we have suggested that the preliminary immunity response in infection by *W bancrofti* is anaphylaxis plus an eosinophilia, and that in circumstances favourable to large releases of filarial toxin the positive complement-fixation reaction is superadded as a further stage of immunization, the clinical result of the increased toxin releases being an 'attack' in types of obstruction where the toxin releases are normally small, such as in blockage of the limb lymphatics, but no attack in circumstances where the lymphatics are still freely open as in chyluria

6 Two cases are referred to showing the effect upon the positive complement-fixation reaction of surgical treatment of the antigenic factor

7 Of four cases of guinea-worm disease three reacted positively, and the positive reaction remained unchanged for long periods The long duration of the positive reaction is probably due to the absence of lymphatic obstruction, bringing our findings in guinea-worm disease into line with Fairley's findings in *L loa* and

our own findings in chyluria As in chyluria, eosinophilia was present in the one case of guinea-worm disease which yielded a negative complement-fixation reaction as well as in those which reacted positively Adequate facilities for the study of guinea-worm disease are not available in Calcutta, but it is suggested that examination of this test will probably not only lead to a valuable means of diagnosis of guinea-worm disease, but will also provide important immunological data which may be applicable to the more complex case of infection with *W bancrofti*

8 From Fairley's results in *L loa* and our own in guinea-worm disease it seems likely that the complement-fixation test by means of Dirofilaria extracts is a group reaction for the worms of the filarial family generally

9 As the clinical manifestations of *W bancrofti* infection are largely those of lymphatic obstruction whereby the antigenic factor is to a progressive extent cut off from the circulation, no serological test could be expected to diagnose all types and stages of the disease, but in a somewhat restricted field the test seems likely to be useful in diagnosis

10 So far as examined, the specificity of the test seems quite satisfactory Apart from guinea-worm disease, no positive reactions were obtained in other helminthic diseases, except in two cases of hookworm infection, in both of which *W bancrofti* infection was also present

11 As we have no method at present of combating the filarial toxin directly, the demonstration that some attacks of lymphangitis are of purely toxic origin is not of immediate therapeutic value, except in so far as it suggests that probably no benefit is to be looked for from vaccine treatment in this type of attack

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TYPES OF TUBERCLE BACILLI CAUSING EXTRA PULMONARY LESIONS IN INDIA

BY

A C UKIL,

Officer-in-Charge, Tuberculosis Inquiry, Indian Research Fund Association, Visiting Physician, Chest Department, Medical College Hospital, Calcutta, Professor of Bacteriology, National Medical Institute, Calcutta

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In an earlier paper (Ukil, 1930) I have tried to show the degree of tuberculization of the people of this country at various age periods I have stated therein that approximately 17 per cent of all cases attending the hospitals are found to suffer from glandular tuberculosis and 13 9 per cent from bone and joint tuberculosis It would be interesting to know about the sources of infection in extra-pulmonary forms of tuberculosis in this country The chief sources of the bovine bacillus are milk and butter from tuberculous cows The work of Taylor (1918) and others indicate that tuberculosis among cattle in India is much rarer than in Europe and America and that tuberculosis of udders is still less common

So far as the morphology, cultural characters and pathological effects produced are concerned, tuberculosis in man and animals, though it may vary in degree, may be considered to be the same disease The consensus of opinion is to regard them as varieties of the same species rather than as distinct organisms We can differentiate three types of bacilli on the basis of their cultural characters, their pathogenic properties and the frequency with which they cause disease in man, cattle and birds

Incidence of bovine infection in man in Europe and America

In Europe and America, a considerable amount of the tuberculosis of childhood has been found to be due to infection with bacilli of the bovine type conveyed

in milk from tuberculous cows Forty per cent of the milch cows in England (Brit Ministry of Health Memo , 1931) react to tuberculin and 42 to 50 per cent of cows slaughtered have been found to be tuberculous According to MacFadyean, 30 per cent of breeding cattle above 3 years and 40 per cent of milking breeds above 3 years in England are tuberculous

Statistics vary as to the incidence of bovine tubercular infection in childhood In continental Europe, it forms about 12 to 15 per cent of all tuberculosis of childhood For fuller information the reader is referred to a recent paper by Bruno Lange (1932) In U S A it varies between 6 to 10 per cent In Scotland it is exceptionally high In England, Dr A Stanley Griffith places the figures as follows —

TABLE I

Variety of tuberculosis	Number of cases	PERCENTAGE OF CASES INFECTED WITH BOVINE TYPE OF TUBERCLE BACILLI		
		0–5 years	5–15 years	All ages
Cervical gland	133	84 0	51 5	48 9
Lupus	168	62 5	53 2	52 4
Scrofulodermia	59	50 0	43 2	35 6
Bone and joint	541	29 4	18 6	18 7
Genito urinary	23			17 4
Meningitis	33	33 3	35 0	27 3
Pulmonary	795			2 6
Post mortem cases	183	29 7	14 3	22 3

Thus, in England a large proportion, in some cases as much as half, of the tuberculous disease in children under 15 is caused by the bovine type of the bacillus It was thought some years ago that there was little or no evidence of bovine infection producing pulmonary tuberculosis in children But recent investigations by Stanley Griffith (1932), Munro and Cumming in England (3 6 per cent of sputa giving positive results) and Bruno Lange in Germany (20 per cent sputa of adults in contact with cattle giving positive results), who have isolated bovine bacilli from cases of phthisis pulmonalis, throw open a new chapter for investigation in different lands This danger will not, however, be great in this country, so long as the bovine infection in cattle remains low

SOURCE OF MATERIALS FOR OUR STUDY

The age incidence of our cases and the nature of materials obtained were as follows —

TABLE II

Source of material	Number of cases	AGE INCIDENCE					
		0–5 years	6–15 years	16–25 years	26–35 years	36–45 years	Above 45 years
Cervical glands	36		5	20	7	3	1
Axillary glands	6	1	4		1		
Inguinal gland	1		1				
Bone and joints	14	1	3	7	3		
Enucleated tonsils	2	Age not known					
Post-mortem gland	1	" " "					

Of the above number 16 were females, the rest males Two were Europeans and the rest Indians

Grouped according to habitation in rural, semi-rural or urban areas, after the classification followed in one of our previous papers (Ukil, 1927), they were as follows —

Rural	16
Semi-rural	7
Urban	37

The enucleated tonsils were sent from the Madanapalle Sanatorium in South India, the details of the cases not being available

TECHNIQUE EMPLOYED

In isolating the strains —In case of pus, a smear examination was first made with Ziehl-Neelsen and Gram stains to determine the presence of tubercle bacilli and secondary organisms respectively A culture was then made on an agar slope and broth to indicate the nature of secondary organisms, if present If the culture remained sterile after 24 hours, the material was directly inseminated on Dorset's egg and potato medium, and a portion inoculated intraperitoneally and subcutaneously (in the right groin) into two guinea-pigs In case secondary organisms were present, the material was treated with 4 per cent NaOH for a variable length of time, the duration of exposure varying with the nature of micro-organisms present

Prior to this a preliminary set of experiments was carried out to find out the time limit of exposure for each kind of micro-organism It was found that a short

exposure varying from 3 to 5 minutes sufficed to kill streptococci, *B pyocyaneus* and *B coli* required an exposure of 10 to 15 minutes, while staphylococci required a fairly long exposure of 30 minutes, and sometimes more With spore-bearing bacteria, a much longer period, as much as 2 to 3 hours, was required to sterilize them This latter was necessary for post-mortem materials Though this was an approximate standard, it was found by experience that different strains of tubercle bacilli resisted differently the action of NaOH, for example, in one case I found a pure growth of tubercle bacilli on Petroff's medium after only 3 minutes' exposure, whereas the tubes inseminated after an exposure longer than 5 minutes and more remained sterile Hence, after this I used to inoculate a number of media after every 5 minutes' exposure up to 30 minutes when aerobic non-sporing organisms were present For inseminating the culture media, it was not found necessary to neutralize the mixture with 4 per cent HCl In this way 18 strains were isolated by direct culture which depends, of course, on the richness of bacilli in the original material

For inoculating into guinea-pigs, the mixture, after exposure, was neutralized with 4 per cent HCl, then centrifuged and washed with physiological saline and the deposit injected The guinea-pigs, if they did not die, were killed after 8 to 10 weeks, and the glands, spleen and liver inseminated on Dorset's medium and potato medium The most successful results as regards culture were obtained from the inguinal and iliac glands

As regards media used in the beginning inspissated beef serum, Petroff, Dorset and potato medium, both glycerinated and non-glycerinated were used, but with subsequent experience it was found that Dorset's and potato media sufficed to give sufficiently successful results

Subsequent cultures —When the primary cultures were obtained, the subsequent cultures were made on Dorset's and potato medium every fortnight, as also on glycerinated and non-glycerinated broth, to study the cultural characters Once they were done, the strains were conserved on Dorset's and glycerinated agar medium No advantage was taken to study the alteration in reaction of liquid media on culture

IN IDENTIFYING THE STRAINS BY ANIMAL INOCULATION

(*a*) *Weighing and emulsification of bacilli* — One milligram of bacilli from the growing margin of a young early culture on Dorset's egg medium was weighed in a small sterile Petri dish and transferred to a thick-walled sterile test-tube with rough-surfaced glass beads, thoroughly shaken to break up the clumps and emulsified by the addition of physiological saline drop by drop till 10 c c were added To control whether gross clumps were present in the emulsion, a smear was made, stained by Ziehl-Neelsen method and examined under the microscope Required quantities were then measured from the emulsion volumetrically

(*b*) *Inoculation into animals*—*Rabbits* Rabbits weighing between 1,200 to 1,800 grammes were employed In the beginning 3 rabbits for *intravenous* inoculation with 1 0 mg , 0 1 mg and 0 01 mg respectively were employed, but later on I substituted the first dose with 10 mg subcutaneously to help in determining the virulence of a possible bovine strain The animals were weighed every week and, if they did not die with in 3 months, were killed after varying periods and autopsied In case of doubt regarding any data experiments were repeated more than once to arrive at a definite conclusion

In autopsying rabbits the naked-eye characters of lesions at the site of inoculation and in lungs, kidneys, tracheo-bronchial glands and other organs were noted and smears were examined for the presence of acid-fast bacilli Histological examinations were resorted to in cases of doubt In many cases the strains were recovered by culture Particular care was taken to find out the presence of intercurrent diseases in case of early death or otherwise

Guinea-pigs All the strains which appeared to be of human type were further inoculated subcutaneously into 3 guinea-pigs weighing between 300 to 400 grammes with 0 1 mg of each strain to find out whether a strain of bacillus fell within the category of a modified human or modified bovine type The examination by autopsy of the guinea-pig is more laborious, inasmuch as not only the regional glands but all the gland groups have to be examined by the naked eye for enlargement and caseation, and verified by a smear examination for acid-fast bacilli but the spleen, liver and lungs have to be similarly examined The loss in weight of the animals was noted weekly till they died In many cases the strains were recovered by culture for purposes of verification

The autopsy findings were recorded in a specially printed schematic diagram for each animal inoculated with different stains for purposes of graphic comparison

Findings

Sixty strains out of 140 materials obtained are included in this study

Cultural characters

Richness of growth—The majority of strains were 'eugonic' and glycerophilic They produced a dry or slightly moist cream-coloured growth on glycerin-potato medium Some, e g , strains 20 P, 22 P and 29 P, produced a distinctly brown colour on growth Some of the strains grew luxuriantly on glycerinated Dorset but not so well on glycerin-potato medium, e g , strains 74 P, 90 P, 92 P Some of the strains were slightly 'dysgonic' but were glycerophilic and produced a cream colour on glycerin-potato medium, e g , strains 26 P, 38 P, 49 P, 50 P, 51 P, 90 P, 96 P, 101 P, 110 P and M 29 Strain P M 6 presented a moist and wrinkled appearance but gave a rich growth on glycerin-potato Strain 125 P is interesting

TABLE III—*contd*

		Intravenous inoculation of rabbits			Subcutaneous inoculation of rabbits with 10 mg		Subcutaneous inoculation of guinea pigs with 0·1 mg		Remarks
		after 0·1 mg	Evidence at autopsy	Result after 0·01 mg	Evidence at autopsy	Result	Evidence at autopsy	Result	Evidence at autopsy	
		K+30 weeks	A few discrete rather big tubercles	K+33 weeks	No lesion on autopsy	D+13 weeks	Collection of caseated pus at site of inoculation No lesion in lungs	D+79—195 days	Caseo necrotic changes in inguinal, pelvic and tracheo bronchial glands Scattered lesions in lungs, liver and spleen	
6	14 P	K+31 weeks	No lesion	K+33 weeks	No lesion	K+20 weeks	Collection of caseated pus at site of inoculation Discrete tubercles in lungs	D+90 days	Collection of pus at site of inoculation Lesions found in inguinal, pelvic, tracheo bronchial and axillary glands, spleen, liver and lungs	
7	16 P	K+33 weeks	Very few big tubercles in lungs A tubo ovarian caseating mass showing T B	K+30 weeks	Very few tubercles in lungs	K+7½ weeks	Local collection of pus only No lesions in lungs	D+74—200 days	Inguinal, pelvic and tracheo bronchial glands involved Rather discrete lesions in spleen, liver and lungs	
8	20 P	K+32 weeks	No lesion	D+6 weeks	A few discrete tubercles	D+10 weeks	Local collection of pus Discrete lesions in lungs	D+80 days	Do *plus* a local collection of pus	

9	21 P	K+23 weeks	Numerous minute tubercles in lungs only	D+14 weeks K+28 weeks	Numerous minute tubercles in lungs only A few discrete tubercles in lungs only	D+16 weeks	Do	D+80—102 days	Do	
10	22 P	K+30 weeks	No lesion	K+32 weeks	No lesion	D+11 weeks	Do	D+95 days	Do	
11	25 P	K+20 weeks	Discrete lesions in lungs	K+20 weeks	Discrete lesions in lungs	K+35 weeks	No lesion, either local or in lungs	D+77—180 days	Involvement of inguinal, pelvic and tracheo bronchial glands and of spleen, liver and lungs Caseous abscess at site of inoculation.	
12	26 P	K+16 weeks	Numerous minute tubercles in lungs	D+9 weeks	Numerous minute tubercles in lungs	D+11 weeks	No other lesion except caseous abscess at site of inoculation.	D+73—78 days	Glandular involvement of almost all groups Lesions in spleen liver and lungs	
13	27 P	K+29 weeks	Numerous minute tubercles in lungs	K+31 weeks	Very few tubercles in lungs	D+9 weeks	Caseous abscess at site of inoculation No other lesion	D+42 days	Involvement of almost all gland groups and of spleen No lesion in lungs or liver	
14	29 P	K+28 weeks	Numerous minute tubercles in both lungs	K+28 weeks	Numerous minute tubercles in lungs	D+10 weeks	Scattered tubercles in lungs	D+144 days	Involvement of inguinal, pelvic and tracheo bronchial glands Scattered tubercles in spleen, liver and lungs Caseous pus at site of inoculation	

D+ = death after K+ = killed after Richness of tubercles in an organ is expressed in grades as + ++, +++

TABLE III—*contd*

Serial number	Number of strain	Intravenous inoculation of rabbits				Subcutaneous inoculation of rabbits with 10 mg		Subcutaneous inoculation of guinea pigs with 0·1 mg		Remarks
		Result after 0·1 mg	Evidence at autopsy	Result after 0·01 mg	Evidence at autopsy	Result	Evidence at autopsy	Result	Evidence at autopsy	
15	36 P	K+16 weeks	Numerous big tubercles in lungs A few tubercles in kidneys	K+16 weeks	Discrete lesions in lungs only		Strain lost			A rabbit inoculated with 1·0 mg intravenously, when killed 15 weeks after, showed numerous tubercles in lungs and a few in kidneys
16	37 P	K+20 weeks	Discrete matter — big tubercles	D+15 weeks	Numerous minute tubercles	D+22 weeks	No demonstrable lesion	D+137 days	Involvement of inguinal, pelvic and tracheo-bronchial glands and of spleen, liver and lungs	
17	38 P	K+24 weeks	Scattered tubercles in lung	K+28 weeks	A few big tubercles in lungs	K+14 weeks	Caseous abscess at site of inoculation Discrete tubercles in lungs	D+125—150 days	Do *plus* caseous abscess at site of inoculation	A rabbit inoculated intravenously with 1·0 mg of bacilli, when killed 24 weeks later, showed numerous tubercles in lungs and a few in kidneys
18	44 P	D+21 weeks	Numerous minute tubercles in lungs Axillary glands showed T B	K+21 weeks	Discrete rather big tubercles in lungs	D+18 weeks	Numerous minute tubercles in lungs Caseous abscess at site of inoculation	D+74—140 days	Caseous abscess at site of inoculation Involvement of inguinal and pelvic glands and of spleen	

19	47 P	D+18 weeks	Numerous small tubercles in lungs	K+24 weeks	Numerous small tubercles in lungs	K+16 weeks	Do	D+200 days	Caseous abscess at site of inoculation Involvement of inguinal pelvic and tracheobronchial glands and of spleen and lungs	With 1 0 mg dose intravenously a rabbit showed a few tubercles in spleen and kidney and axillary gland, besides numerous ones in lungs
20	48 P	K+18 weeks	Numerous small tubercles in lungs	K+19 weeks	A few tubercles in lung	D+7¹ weeks	Do	D+120 days	Involvement of inguinal pelvic and tracheobronchial glands. No lesion in spleen, liver or lungs	
21	49 P	K+21 weeks	A few minute tubercles in lungs	K+22 weeks	No demonstrable lesion in lungs or in other organs	D+8 weeks	Caseous abscess at site of inoculation Numerous minute tubercles in lungs	D+57—101 days	Caseous abscess at site of inoculation Involvement of inguinal pelvic and tracheobronchial glands and of [illegible]	
22	50 P	K+21 weeks	Discrete tubercles in lungs	K+26 weeks	Discrete tubercles in lungs	D+7¹ weeks	Caseous abscess at site of inoculation No lesion in lungs	D+116 days	Do	Intravenous inoculation of 1 0 mg of bacilli showed in a rabbit, when killed 20 weeks after tubercles in lungs and kidneys
23	51 P	K+16 weeks	Discrete greyish tubercles in lungs	K+24 weeks	Very few tubercles in lungs	D+9 weeks	Caseous abscess at site of inoculation Numerous small tubercles in lungs	D+72 days	Do *plus* involvement of axillary glands	

D+ = death after K+ = killed after Richness of tubercles in an organ is expressed in grades as + ++ +++

TABLE III—*contd*

Serial number	Number of strain	Intravenous inoculation of rabbits				Subcutaneous inoculation of rabbits with 10 mg		Subcutaneous inoculation of guinea pigs with 0·1 mg		Remarks
		Result after 0·1 mg	Evidence at autopsy	Result after 0·01 mg	Evidence at autopsy	Result	Evidence at autopsy	Result	Evidence at autopsy	
24	53 P	K+22 weeks	Numerous minute tubercles in lungs	D+11 weeks	Numerous minute tubercles in lungs	D+12 weeks	Caseous abscess at site of inoculation Numerous small tubercles in lungs	D+150 days	Involvement of inguinal, pelvic and tracheobronchial glands and of spleen and lungs	Intravenous inoculation of 1·0 mg into rabbit showed, when killed 20 weeks after, a few tubercles in kidneys and numerous tubercles in lungs
25	59 P	D+9 weeks	Numerous minute tubercles in lungs	K+19 weeks	No lesion		Strain lost			Rabbits inoculated intravenously with 1·0 mg of bacilli showed, when killed after 7 weeks and 16 weeks, only minute tubercles in lungs
26	62 P	K+32 weeks	No lesion	K+32 weeks	No lesion	D+15 weeks	Only caseous abscess at site of inoculation	D+58 days	Caseous abscess at site of inoculation Involvement of almost all groups of glands and of spleen	With 1·0 mg intravenously into rabbits very few tubercles were found when killed after 25 weeks

27	64 P	K+24 weeks	Fair number of big tubercles (caseo necrotic) in lungs	D+10 weeks	Numerous *minute* tubercles in lungs	D+12 weeks	Caseous abscess at site of inoculation Numerous minute tubercles in lungs	D+90—110 days	Caseous abscess at site of inoculation Involvement of inguinal pelvic and tracheo bronchial glands and of spleen, liver and lungs	
28	67 P	D+29 weeks	Numerous minute tubercles in lungs	K+32 weeks	A fair number of minute tubercles in lungs	D+14 weeks	Do	D+128 days	Do	
29	68 P	D+5 weeks	Numerous minute tubercles in lungs	K+24 weeks	A fair number of minute tubercles in lungs	D+9 weeks	Only caseous abscess at site of inoculation	D+50—128 days	Do	
30	74 P	K+24 weeks	Numerous caseo necrotic tubercles in lungs A caseated gland in pelvis and chest wall and a few tubercles in kidneys and subperitoneal surface of large intestine	K+24 weeks	No lesion	D+9 weeks	No lesion	D+64—100 days	Do	
31	75 P	K+30 weeks	A few tubercles in lungs	K+30 weeks	A few tubercles in lungs		Strain lost			
32	76 P	K+29 weeks	A fair number of big tubercles	K+29 weeks	Fair number of minute tubercles	D+7½ weeks	Only caseous abscess at site of inoculation	D+106—200 days	Caseous abscess at site of inoculation Involvement of lungs liver spleen and almost all gland groups	A few tubercles also in kidneys with 10 mg intravenously and killed after 17 weeks

D+ = death after K+ = killed after Richness of tubercles in an organ is expressed in grades as + ++, +++

TABLE III—*contd*

Serial number	Number of strain	Intravenous inoculation of rabbits				Subcutaneous inoculation of rabbits with 10 mg		Subcutaneous inoculation of guinea pigs with 0 1 mg		Remarks
		Result after 0 1 mg	Evidence at autopsy	Result after 0 01 mg	Evidence at autopsy	Result	Evidence at autopsy	Result	Evidence at autopsy	
33	82 P	K+30 weeks	A fair number of tubercles in lungs	K+30 weeks	A few tubercles in lungs	D+8 weeks	Caseous abscess at site of inoculation Fair number of tubercles in lungs	D+73—140 days	Caseous abscess at site of inoculation Involvement of spleen, liver and lungs and of inguinal pelvic and tracheo bronchial glands	
34	85 P	D+17 weeks	A fair number of minute tubercles in lungs	K+30 weeks	Very few tubercles in lungs	D+30 weeks	A fair number of tubercles in lungs	D+164 days	Caseous abscess at site of inoculation Involvement of inguinal, pelvic and tracheo bronchial glands and of spleen	
35	87 P	K+23 weeks	Lungs—a large number of minute tubercles A parietal abscess on abdominal wall showing tubercle bacilli	D+9 weeks	A fair number of minute tubercles in lungs	D+24 weeks	Only a caseous abscess at site of inoculation	D+230 days	Caseous abscess at site of inoculation Involvement of inguinal, pelvic and tracheo bronchial glands and of spleen and lungs	
36	88 P	K+23 weeks	Lungs—fair number of tubercles Kidney—a few tubercles	D+16 weeks	Lungs—a few tubercles	D+14 weeks	Do	D+230 days	Do	

37	89 P	K+23 weeks	Lungs—fair number of tubercles	K+23 weeks	Lungs—fair number of tubercles	D+42 weeks	Caseous abscess at site of inoculation A few discrete tubercles in lungs	D+61 days	Do	Intravenous inoculation of a rabbit with 10 mg showed, when death ensued after 5 weeks a few tubercles in kidneys and numerous minute ones in lungs
38	90 P	K+14 weeks	Lungs—large number of tubercles Kidneys—a fair number of tubercles	K+14 weeks	Lungs—a few tubercles	D+8 weeks	Caseous abscess at site of inoculation	D+140 days	Caseous abscess at site of inoculation Involvement of inguinal, pelvic and tracheo bronchial glands and of spleen	
39	92 P	K+14 weeks	Lungs—fair number of tubercles	K+14 weeks	Lungs—fair number of tubercles Kidney—two tubercles	D+9 weeks	Caseous abscess at site of inoculation Fair number of tubercles in lungs	D+75 days	Involvement of inguinal pelvic and tracheo bronchial glands and of lungs liver and spleen	Intravenous inoculation of 1 0 mg in rabbit when died after 14 weeks showed a fair number of tubercles in kidneys in addition a large number in lungs
40	93 P	D+13 weeks	Lungs +++ Kidney—solitary tubercle	D+12 weeks	Lungs +++	D+15 weeks	Lungs ++	D+63—125 days	Do *plus* caseous abscess at site of inoculation	
41	95 P	K+23 weeks	Lungs—few tubercles	K+23 weeks	Lungs +++	D+11 weeks	Caseous abscess at site of inoculation	D+60 days	Caseous abscess at site of inoculation Involvement of inguinal and pelvic glands and of spleen	

D+ = death after K+ = killed after Richness of tubercles in an organ is expressed in grades as +, ++ +++

TABLE III—*contd*

Serial number	Number of strain.	Intravenous inoculation of rabbits				Subcutaneous inoculation of rabbits with 10 mg		Subcutaneous inoculation of guinea pigs with 0 1 mg		Remarks
		Result after 0 1 mg	Evidence at autopsy	Result after 0 01 mg	Evidence at autopsy	Result	Evidence at autopsy	Result	Evidence at autopsy	
42	96 P	K+28 weeks	Lungs ++ minute tubercles	D+16 weeks	Lungs—b i g g e r tubercles +++	D+11 weeks	Caseous abscess at site of inocula tion Lungs ++	D+97—140 days	Caseous pus at site of inoculation Involvement of inguinal, pelvic and t r a c h e o bronchial glands	Rabbit died 6 weeks after intravenous inoculation with 1 0 mg showing numerous tuber cles in lungs and 3 in kidney
43	101 P	K+16 weeks	Lungs +++	D+10 weeks	Lungs ++	D+20 weeks	Caseous abscess at site of inocula tion	D+84 days	Caseous abscess at site of inocula tion I n v o l v e ment of inguinal, pelvic and tra c h e o bronchial glands and spleen and lungs	
44	103 P	D+7 weeks	Lungs +++ Kidneys ++	K+16 weeks	Lungs +++		*Strain lost*			*Rabbit* inoculated intr a v e n o u s l y with 1 0 mg died 16 weeks later showing numer- ous tubercles in lungs and a fair number in kidney
45	104 P	K+22 weeks	A few tubercles in lungs Caseated pus in right tes tes	K+22 weeks	Very few tubercles in lungs	D+26 weeks	Caseous abscess at site of inocula tion	D+125 days	Caseous pus at site of inocula tion Involve ment of almost all gland groups and of sp'een and lungs	

46	105 P	D+10 weeks	Lungs ++	D+10 weeks	Lungs +	D+21 weeks	Caseous pus at site of inoculation Lungs ++	D+95 days	Caseous pus at site of inoculation Involvement of almost all gland groups.	
47	107 P	D+18 weeks	Lungs ++	D+10 weeks	Lungs +	D+14 weeks	Caseous pus at site of inoculation	D+75 days	Caseous pus at site of inoculation Involvement of inguinal and pelvic glands and of spleen	
48	110 P	D+19 weeks	Lungs ++	D+12 weeks	Lungs +	D+27 weeks	Caseous abscess at site of inoculation	D+90–207 days	Caseous abscess at site of inoculation Involvement of inguinal pelvic and tracheobronchial gland group and of spleen liver and l	
49	121 P	D+28 weeks	Lungs ++	K+32 weeks	Lungs ++	D+19 weeks	Caseous abscess at site of inoculation	D+74 days	Do *plus* involvement of axillary glands	
50	124 P	D+30 weeks	Lungs ++	D+14 weeks	Lungs +	D+21 weeks	Caseous abscess at site of inoculation Lungs ++	D+57—140 day	Caseous pus at site of inoculation Involvement of inguinal pelvic and tracheobronchial glands and of spleen and lungs	Rabbit inoculated with 1·0 mg died 21 weeks later and showed a few tubercles in kidneys and lungs +++

D+ = death after K+ = killed after Richness of tubercles in an organ is expressed in grades as + ++ +++

TABLE III—*concld*

Serial number	Number of strain	Intravenous inoculation of rabbits				Subcutaneous inoculation of rabbits with 10 mg		Subcutaneous inoculation of guinea pigs with 0 1 mg		Remarks
		Result after 0 1 mg	Evidence at autopsy	Result after 0 01 mg	Evidence at autopsy	Result	Evidence at autopsy	Result	Evidence at autopsy	
51	125 P	D+14 weeks	Lungs +	K+21 weeks	No demonstrable lesion	D+7½ weeks	Caseous abscess at site of inoculation	D+100 days	Caseous abscess at site of inoculation Involvement of inguinal, pelvic and tracheobronchial glands and of spleen and lungs	
52	126 P	D+4 weeks	Lungs +++	D+13 weeks	Lungs ++	D+17 weeks	Small caseated abscess at site of inoculation	D+80 days	Involvement of inguinal, pelvic and tracheobronchial glands and of spleen, liver and lungs (rather discrete)	
53	128 P	D+24 weeks	Lungs +	K+16 weeks	No demonstrable lesion	D+7½ weeks	Caseous abscess at site of inoculation.	D+102 days	Caseous abscess at site of inoculation Almost all gland groups involved as also the spleen, liver and lungs in a discrete way	Rabbit receiving a dose of 1 0 mg intravenously died 5 weeks later and showed a fair number of tubercles in kidneys and a large number in lungs
54	132 P	D+13 weeks	Lungs +++	K+72 weeks	No demonstrable lesion	D+9 weeks	Caseous abscess at site of inoculation Lungs +++	D+90 days	Caseous abscess at site of inoculation Almost all gland groups involved as also the spleen, liver and lungs in a discrete way	

55	136 P	K + 32 weeks	Lungs +++	K+24 weeks	Very few tubercles in lungs	K+33 weeks	Caseous abscess at site of inoculation Lungs ++	D+125 days	Do	
56	137 P	K + 18 weeks	Lungs ++ Liver +	K+18 weeks	Lungs +	K+18 weeks	Caseous abscess at site of inoculation	K+135 days	Caseous abscess at site of inoculation Involvement of inguinal and pelvic glands spleen, liver and lungs	
57	140 P	K + 18 weeks	Lungs ++ Rt. testes +++	K+18 weeks	Lungs ++	K+18 weeks	Caseous abscess at site of inoculation Lungs + Liver +	D+45—135 days	Caseous abscess at site of inoculation Involvement of almost all gland groups and of spleen, liver and lungs	
58	P M. 6	K+22 weeks	Lungs ++	K+22 weeks	No demonstrable lesion	D+20 weeks	Caseous abscess at site of inoculation	D+140 days	Do	
59	M. 16	D+24 weeks	Lungs ++	D+32 weeks	Lungs ++	D+12 weeks	Caseous abscess at site of inoculation	D+112 days	Caseous abscess at site of inoculation Involvement of inguinal, pelvic bronchial glands and of spleen	
60	M 29	K+40 weeks	Lungs +++ Kidneys + Pelvic abscess +	D+15 weeks	Very few tubercles in lungs.	D+20 weeks	Caseous abscess at site of inoculation	D+82 days	Involvement of almost all gland groups and of spleen liver and lungs	

D+ = death after K+ = killed after Richness of tubercles in an organ is expressed in grades as +, ++, +++

beyond 70 days—the maximum fatal time limit for bovine strains Among those dying earlier, none seemed to show so extensively progressive caseo-necrotic changes as are found after inoculation with known bovine strains, of which several controls were kept

Discussion

The question of determining the source of extra-pulmonary tubercular infection in man in India is important, in presence of the fact that cattle are much less infected with tuberculous disease than in Europe or America The accepted figure in India is only about 3 to 3 5 per cent, a very low figure compared with that in Europe, though the systematic examination of carcasses for tuberculous disease has not yet been well carried out in all parts of India Latterly Soparkar (1927) found 14 per cent of cattle infected at Ferozepur and Lahore (Punjab), a figure very nearly approaching that prevailing in some parts of Europe There are evidences that, though Indian milch cattle possess a relative immunity against tuberculosis so long as they are kept in fresh air and sunshine (Soparkar, 1926), this barrier seems to give way in presence of massive infection and when they are confined in sheds away from fresh air and sunlight, as has recently happened at the Madras Corporation cattle sheds Bovine tuberculosis in man must be considered to be rare here when it is remembered that all classes of people are accustomed to drink milk boiled, very often more than once, at home

In my series of 60 cases, all the strains have proved to be human in type Out of Soparkar's (1925) 48 strains isolated from extra-pulmonary sources, all were reported to belong to the human type Soparkar (1927) has, however, reported the isolation of 2 bovine strains (verbally communicated) and one avian strain from cases of adenitis in man He has further isolated (verbally communicated) two human strains from cattle in the Punjab I am not conversant with the habits of the people in the Punjab, but it is noteworthy that the Tuberculosis Research Committee of South Africa (1932) have found that infection of pigs in Johannesburg is fairly common from human tubercle bacilli, probably because the pigs are fed there largely upon refuse food material obtained from the mine compounds, pointing to the possibility of contamination of the food with human tubercle bacilli They have further found all the 50 strains from human sources isolated and studied by them to belong to the human type

As regards the point whether the strains isolated could possibly have been modified bovine bacilli of low virulence, the evidence obtained from rabbit and guinea-pig inoculation tests is clearly against such a view For the moment, therefore, extra-pulmonary tubercular lesions in India must be considered to be due, to an overwhelming extent, to the human type of the bacillus There is always, however, the possibility of an increased incidence of the bovine infection, if in course of time, an increasingly large number of cattle become infected and the

people change their habits of drinking boiled milk This possibility, however, seems to be remote

It may be argued by European workers that the number of cases in children (in whom, for example, Stanley Griffith has found 54 2 per cent of bovine infection in England and Scotland) studied by us is comparatively small and that perhaps a greater percentage of bovine infection would be found in children of this age group But, as I have shown elsewhere, the maximum incidence of pulmonary and extra-pulmonary forms of tuberculosis in this country occurs about the same age period, viz , 25th year, and that, owing to a less perfect immunization of the population than in Europe, a young adolescent of 15 to 20 years here probably corresponds to a child of 5 to 10 years in more urbanized regions of Europe Hence, the data presented may be taken as a correct estimate of the present position of bovine infection in man in this country

The route of entry of the bovine bacillus into human body is thought to be almost exclusively through the alimentary tract by the ingestion of infected milk Though these bacilli may localize in various parts of the body in childhood, they are capable of producing *phthisis pulmonalis* in adult life, as has been suggested by Griffith and Bruno Lange (1932) In India we must look to other sources of alimentary infection, e g , swallowing of food and drink contaminated with human sputa, etc , which appear to be probable from the habits of the people prevailing in various parts of India We are not yet in a position to state to what extent alimentary infection plays the rôle in producing extra-pulmonary and other lesions from human sources in this country

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